# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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## Supplementary Appendix

**Title: Epidemiology of Human Infections with Avian Influenza A(H7N9) Virus in China**

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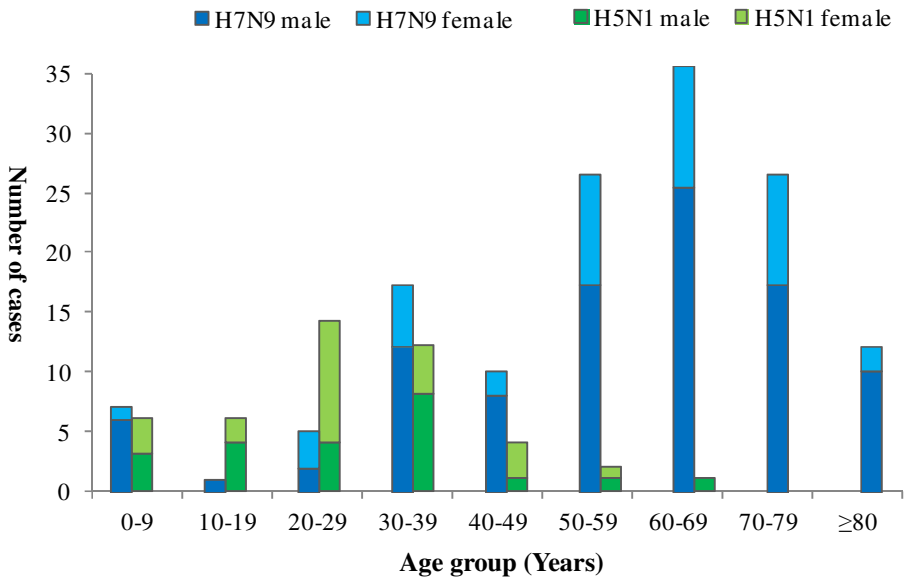
**Supplementary Figure S1. Age and gender distribution of 139 confirmed H7N9 cases as of December 1, 2013 compared with 45 confirmed H5N1 cases in China***




*Unpublished data, China CDC 2013, including one retrospectively-diagnosed male case in 2003, and one female case identified in Hong Kong, SAR, China in 2010.

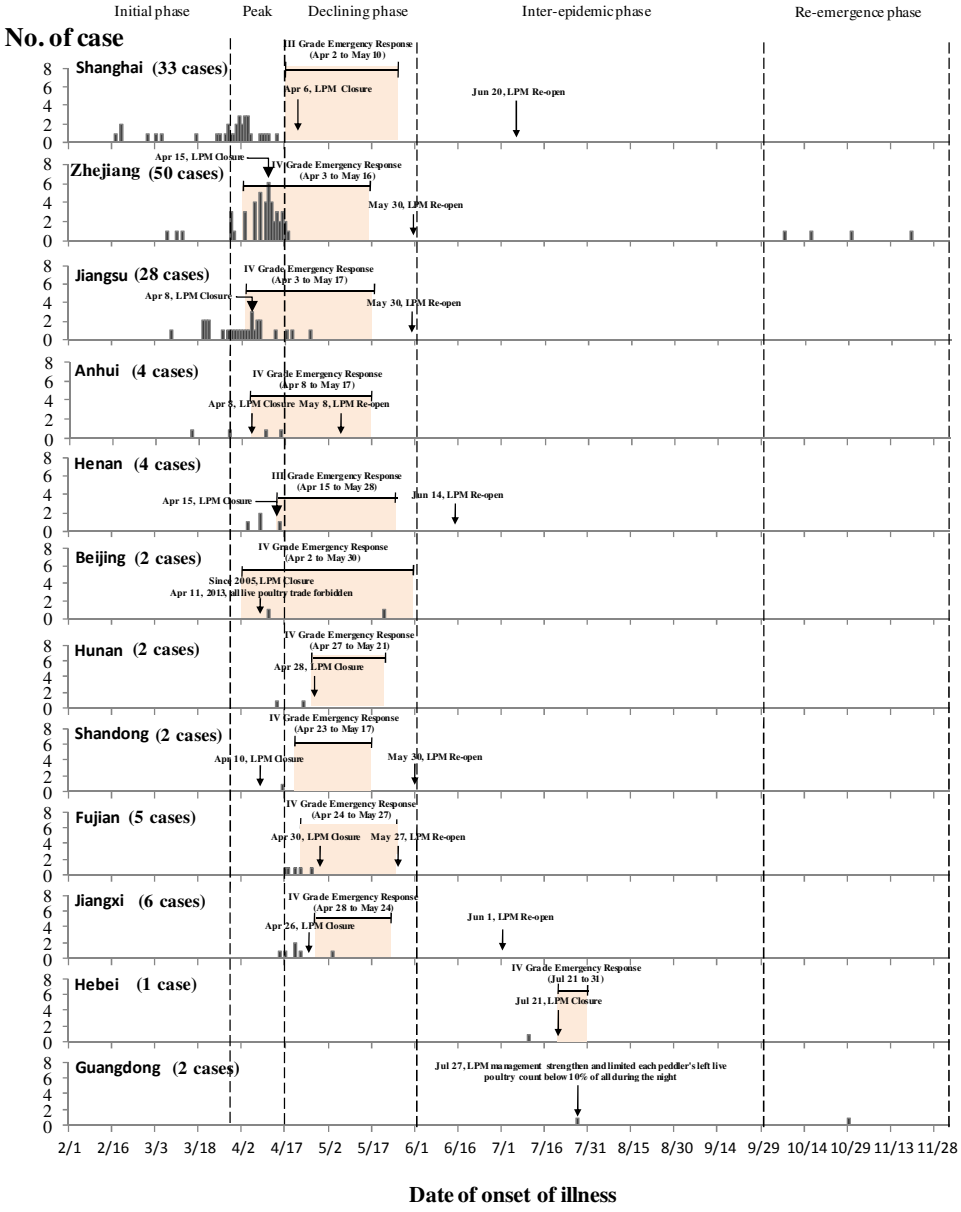
**Supplementary Table S1. Estimated incubation period of 23 human cases of avian influenza A(H7N9) virus infection, China***

| Exposure data | Case-patients with exposure to market and poultry | Case-patients with exposure to market only | Case-patients with exposure to poultry only | Case-patients with exposure to suspected case only | All case-patients |
|---|---|---|---|---|---|
| No. case-patients with exposures on multiple days | 4 | 1 | 4 | 2 | 11 |
| Overall median incubation period, d (range) | 5.5 (5.5-5.5) | 5.5 | 5.5 (5.5-5.5) | 5.25 (4-6.5) | 5.5 (4-6.5) |
| Median of minimum incubation period, d (range) | 1 (1-1) | 1 | 1 (1-1) | 1.5 (0-3) | 1 (0-3) |
| Median of maximum incubation period, d (range) | 10 (10-10) | 10 | 10 (10-10) | 9 (8-10) | 10 (8-10) |
| No. case-patients with single known exposure | - | 10 | 2 | - | 12 |
| Overall median incubation period, d (range) | - | 6 (1-10) | 4 (1-7) | - | 6 (1-10) |
| All case-patients | 4 | 11 | 6 | 2 | 23 |

| | | | | | |
|---|---|---|---|---|---|
| Overall median incubation period, d (range) | 5.5 (5.5-5.5) | 6 (1-10) | 5.5 (1-7) | 5.25 (4-6.5) | 5.5 (1-10) |
| Overall median of minimum incubation period, d (range) | 1 (1-1) | 6 (1-10) | 1 (1-1) | 1.5 (0-3) | 1 (0-10) |
| Overall median of maximum incubation period, d (range) | 10 (10-10) | 6 (1-10) | 10 (1-10) | 9 (8-10) | 7.5 (1-10) |

* The method of estimating incubation periods for H7N9 was based upon methods used for H5N1 as previously described.[1]

**Supplementary Figure S2. Date of onset of illness in 139 patients with confirmed H7N9 virus infection and control measures, according to province or municipality in China**




* LPM: live poultry market. The colored area represents the duration of emergency response in each province.

**Section 1. Case definitions**

The case definitions for suspected and confirmed human infections with novel avian influenza A(H7N9) virus were based upon the H5N1 case definitions as recommended by the World Health Organization (WHO) in 2006.[2] A suspected H7N9 case was defined as a person presenting with unexplained acute lower respiratory illness with fever (≥38 ℃) and cough, shortness of breath or difficulty breathing or infiltrates or evidence of an acute pneumonia on chest radiograph plus evidence of respiratory failure (hypoxemia, severe tachypnea), and (a) positive laboratory confirmation of an influenza A virus infection but insufficient laboratory evidence for H7N9 virus infection because of lack of specimens or (b) epidemiologically-linked to a confirmed H7N9 case, but without any respiratory specimens available for H7N9 testing. Beginning April 3rd, 2013, specimens from outpatients with influenza-like illness (ILI) presenting to sites of the Chinese ILI sentinel surveillance system were tested for H7N9 virus by real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. A confirmed H7N9 case was defined as a patient with ILI or a suspected case with respiratory specimens that tested positive for H7N9 virus by any of the following: isolation of H7N9 virus or positive results by rRT-PCR assay for H7N9, or a fourfold or greater rise in antibody titer for 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 two weeks later (Supplementary Appendix 2).

**Section 2. H7N9 laboratory assays**

RNA was extracted from specimens with the RNeasy mini kit (Qiagen, Valencia, CA, USA) as per the manufacturer's protocol and tested by real-time RT-PCR with H7N9-specific primers and probes as previously described.[3] The specific sequences have been published on the WHO website at http://www.who.int/influenza/gisrs_laboratory/a_h7n9/en/. These assays were done in biosafety level (BSL) 3 facilities at Provincial CDC and NIC of China CDC. Respiratory specimens were inoculated in amniotic cavities of pathogen-free embryonated chicken eggs for viral isolation in enhanced BSL 3 facilities at the NIC of China CDC.

H7N9 serological testing was done by modified hemagglutinin inhibition assay using turkey red-blood-cells in BSL 2 conditions at the NIC of China CDC. Antigens for the assays were produced from the A/Anhui/1/2013 (H7N9) virus isolated from a confirmed case in Anhui Province.[3] An individual was considered to be seropositive for H7N9 if a four-fold or greater rise in H7N9 virus antibody was detected by testing paired acute and convalescent sera.

**Section 3. Close contact identification and follow-up**

We defined close contacts of confirmed H7N9 cases as described previously for H5N1 field investigations.[4] Close contacts were defined as individuals known to have been within 1 m, or had direct contact with respiratory secretions or faecal material, of a patient with confirmed H7N9 virus infection any time from the day before the onset of illness to when the case was isolated in the hospital. Antiviral chemoprophylaxis was not provided to close contacts. Close contacts were monitored daily for seven days by telephone or in-person for development of fever or any respiratory symptoms after their last known exposure to a confirmed H7N9 case-patient. Throat swabs were collected from close contacts with fever or any respiratory illness symptoms on a median 1 day (range: 0-8) after their illness onset and placed into sterile viral transport media for H7N9 virus testing at the China CDC. Symptomatic close contacts were recommended to receive oseltamivir treatment if oseltamivir was available. Demographic information and data were collected on use of personal protective equipment, use of oseltamivir, illness symptoms, and potential H7N9 virus exposures (eg, poultry contact, visiting poultry markets, contact with wild or pet birds, and contact with individuals with febrile respiratory symptoms) during the 2 weeks before the last known exposure to an ill confirmed H7N9 case-patient.

**Section 4. Findings of Four family cluster investigations**

**Shanghai cluster 1 (father and sons)**

One family cluster was identified with two confirmed and one suspect H7N9 cases. The confirmed cases were in a 69-year-old man and his father, an 87-year-old retired man, a resident of Shanghai City. The suspect case, aged 57 years, was the first ill person in the household and developed high fever (maximum temperature 41ºC), cough, sputum production, chills and nausea on Feb 11th (Figure 2). After the suspect case patient became ill, his brother (confirmed case 1) and the father (confirmed case 2) had close unprotected contact with him, including eating together, providing care, and accompanying him to seek medical care before his hospitalization. The suspect case patient was hospitalized on Feb 20th, diagnosed with pneumonia, placed on mechanical ventilation and isolated in an intensive care unit the next day. He died of ARDS and multi-organ failure on Feb 28th. The throat swab collected from him on Feb 26th tested negative for H7N9 by rRT-PCR, and no additional specimens were available for H7N9 testing because of his death. Confirmed case 1 developed fever (40 ºC), cough, sputum production, nausea and vomiting on Feb 19th, was admitted to hospital on Feb 25th with a diagnosis of pneumonia, and was isolated the next day. A throat swab collected from him on Feb 26th tested negative for H7N9 by rRT-PCR. He improved and was discharged on Mar 13th. Paired serum specimens collected 7 and 46 days after illness onset were tested by turkey red blood cell hemagglutinin inhibition assay and demonstrated a four-fold rise in antibody titer (HI titer <10 and 80, respectively), indicative of serological confirmation of H7N9 virus infection. The

87-year old father (confirmed case 2) developed cough and sputum production on Feb 19th and had high fever (40.2 ºC) on Feb 24th, was admitted to hospital on Feb 25th with a diagnosis of pneumonia, and isolated the next day. A throat swab collected on Feb 26th tested positive for H7N9 viral RNA by rRT-PCR. He died on Mar 4th. The suspect case and his father (confirmed case 2) lived together and the brother (confirmed case 1) lived with his wife nearby. All three cases did not raise poultry or other animals, and did not bring live poultry into their home. None of the three cases in this family cluster had any direct contact with sick or dead poultry. The suspect case had visited a live poultry market, purchased a well-appearing chicken, observed the slaughtering process, brought the freshly killed chicken home, prepared, cooked and ate the chicken within 2 weeks before his illness onset, but the exact exposure date was unclear because he was severely ill and died before he could be interviewed. A total of 29 close contacts of the two confirmed cases, including 16 household members and relatives, 7 healthcare workers, and 6 social contacts, did not develop any illness during the 7-day monitoring period.

**Wuxi, Jiangsu cluster (father and daughter)**

Another family cluster was identified with two confirmed cases in a father (confirmed case 1) and his daughter (confirmed case 2) who lived together with case 2's mother, husband and son in the same house. The father (case 1) was a 60-year-old man with hypertension, who developed fever, cough and shortness of breath on March 8th, and was admitted to hospital three days later and isolated in an intensive care unit on Mar

15th, and died of multiple organ dysfunction (MODS) on May 4th (Figure 2). The daughter (case 2), a 32-year-old woman without any underlying medical conditions, developed illness onset on Mar 21st with fever (40 ºC) and cough, was hospitalized on Mar 24th, isolated in an intensive care unit on Mar 28th, and died of MODS on April 24th. The father's throat swab collected on Mar 27th was positive for influenza A, but negative for H7N9 by rRT-PCR. An additional throat swab collected on Mar 31st was positive for H7N9 by rRT-PCR and a single serum specimen collected on the same day (30 days after illness onset) tested seropositive for H7N9 virus antibodies with a titer of 320 by HI assay. Testing of tracheal secretion specimens collected from the daughter on Mar 31st confirmed H7N9 virus infection by rRT-PCR. After the father became ill, his daughter had close unprotected contact with him, including eating together, providing care, and accompanying him to seek medical care before his hospital admission. She also provided unprotected bedside hospital care for her father during March 11th-15th. After isolation, the father developed diarrhea. The confirmed case had washed her father's diarrhea-soiled underwear on March 18th while wearing gloves. The father had visited a live poultry market within 2 weeks before his illness onset but the exact date of exposure is unclear. The daughter did not raise poultry or animals at home, and had not had any animal exposures (had not brought live poultry into the home or visited a wet poultry market or had any direct or indirect contact with poultry or pigs). A total of 43 close contacts, including 38 healthcare personnel, of the two confirmed cases were monitored for 7 days. Among their household contacts, case 2's husband developed fever (37.5 ºC) on March 24th, the same day of the last

known exposure to a confirmed case, and recovered on the 10th day after illness onset. Throat specimens collected from case 2's husband on March 28th and 31st tested negative for H7N9 by rRT-PCR. Additional information on this cluster was described previously.[5]

**Shanghai cluster 2 (wife and husband)**

The third family cluster was identified with two confirmed cases in Shanghai. The two confirmed cases were in a 51-year old woman with a thyroid tumor and her 56-year old husband, both retired residents of Shanghai Municipality. The wife (confirmed case 1) developed feverishness, chills and fatigue on March 27th and visited the community hospital for intravenous antibiotic treatment with a diagnosis of pneumonia during March 28th-30th, and a fever of 39 ℃ (Figure 2). Her respiratory symptoms worsened on April 1st when she visited a prefecture hospital. She was admitted the next day with a diagnosis of severe pneumonia and ARDS, and died of respiratory failure on April 3rd. A respiratory tract aspirate specimen collected before her death on April 3rd was positive for H7N9 viral RNA by rRT-PCR. Her husband, (confirmed case 2), who was previously healthy, developed fatigue on April 1st, runny nose on April 2nd, and had a fever (38.2 ℃) when he visited a prefectural hospital on April 3rd. His illness worsened and was admitted the next day, deteriorated rapidly during the hospitalization, and died of ARDS and multi-organ failure on June 26th. A throat swab collected on April 10th was positive for both influenza A(H1N1)pdm09 and H7N9 by rRT-PCR. After the wife became ill, her husband had close unprotected

contact with her, including eating together, providing care, and accompanying her to seek medical care before her hospital admission. The husband also provided unprotected bedside hospital care for his wife during April 2nd-3rd. The wife had visited a live poultry market within one week before her illness onset but the exact date of exposure was unclear. The husband did not raise poultry or animals at home, and had not had any animal exposures (had not brought live poultry into the home or visited a wet poultry market or had any direct or indirect contact with poultry or pigs). A total of 18 close contacts, including 12 healthcare personnel, of the two confirmed cases were monitored for 7 days. Among their household contacts, the wife's sister developed a fever (37.5 ºC) on April 4th, the second day of the last known exposure to a confirmed case, was admitted in hospital with a diagnosis of pneumonia on April 5thand started on oseltamivir treatment. A throat swab collected on April 5th was negative for H7N9 by rRT-PCR, and she was discharged on the 5th day after illness onset.

**Zaozhuang, Shandong cluster (father and son)**

The fourth family cluster was identified with two confirmed cases in Shandong. The first case occurred in a previously healthy 36-year-old man (confirmed case 1), who was a resident of Zaozhuang City, Shandong Province. He developed fever (38.8 ºC) and cough on April 16th which worsened on April 19th. He visited a private clinic for intravenous antibiotic treatment from April 18th to 20th. On the morning of April 21st he was admitted with a diagnosis of pneumonia, and was placed on mechanical

ventilation and isolated in an ICU the next day. Oseltamivir treatment was started on the same day. He recovered and was discharged on May 16th. His throat swab collected on April 21st tested positive for H7N9 viral RNA by rRT-PCR assay in the Shandong provincial CDC laboratory on April 22nd and was confirmed by the National Influenza Center of the Chinese Center for Disease Control and Prevention on April 23rd. His 4-year-old son (confirmed case 2) developed a temperature of 37.3 ºC and cough on April 27th during the monitoring period for close contacts, and was admitted to hospital the next day. He recovered and was discharged on May 6th. A throat swab collected on April 27th tested positive for H7N9 viral RNA by rRT-PCR assay in the Shandong provincial CDC laboratory on April 28th. The father and his son lived together with the wife and two daughters. The family did not raise poultry or other animals, and did not bring live poultry into their home. Neither of the two cases in this family cluster had any direct contact with poultry. Both cases had not visited a live poultry market within 2 weeks before onset of illness. There was a chicken coop about ten meters in front of the building where the family lived. The family's car was usually parked next to a chicken coop. During the 2 weeks before onset of illness, the father (confirmed case 1), drove the car with his wife at least twice per day. After the father became ill, his son had direct and very close unprotected contact with him, including hugging, sitting, eating, and sleeping together. During April 18th-20th, he accompanied his sick father and well mother each day to the private clinic where his father received treatment. The 11 close contacts of the father and son, including 3 household members (case 1's wife and two daughters), 4 relatives (case 1's mother,

mother-in-law, brother and brother's wife), 2 friends and 2 health care workers did not develop any illness during 7-day monitoring period.

**Section 5. Description of 28 ill close contacts during the 7-day surveillance period**

As of December 1, 2013, excluding confirmed cases that were close contacts of index cases in the four family clusters, all 2675 close contacts of 139 confirmed H7N9 cases had completed a 7-day observation period. Among them, 28 (1%) close contacts (nine household members, one medical intern, one patient of same ward, and 17 health care workers) developed acute respiratory symptoms or weakness or upper respiratory illness during the 7-day surveillance period, and all tested negative for H7N9 virus by rRT-PCR. Of two ill household contacts identified in each of two clusters (Jiangsu, and Shanghai cluster 2), both tested negative for H7N9 by rRT-PCR (Section 4). One ill household member, the niece of a confirmed case in Jiangsu, developed fever (37.3 ºC) on the second day of the last known exposure to a confirmed case. One ill household member, the wife of a confirmed case in Henan, developed fever and cough on the fifth day of the last known exposure to a confirmed case, and received oseltamivir treatment on the day of illness onset. The other two ill household members, the daughter-in-law and grandson of a confirmed case in Hunan, developed fever (daughter-in-law and grandson) and diarrhea (grandson) on the same day and fourth day of the last known exposure to a confirmed case, respectively. One ill household member, the granddaughter of a confirmed case in Hunan, developed fever on the third day of the last known exposure to a confirmed case. One ill household member, the son of a confirmed case in Guangdong, developed fever on the second day of the last known exposure to a confirmed case. Another ill household member, the brother of a confirmed case in Guangdong, developed cough two days before the

onset of illness of a confirmed case, but a throat swab was not collected until 8 days after illness onset and six days after the confirmed case's illness onset. The intern was the doctor for a confirmed case in Anhui and provided care for the case patient in the intensive care unit while wearing personal protective equipment, including N95 respirator, gloves and gowns. He developed fever on the last known day of exposure to the confirmed case patient, and recovered the next day. One patient hospitalized in the same room with a confirmed case in Zhejiang developed slight fever after exposed to the confirmed case patient. 15 health care worker contacts that provided care for a confirmed case in Zhejiang developed cough and sore throat during the 7-day monitoring period. A doctor provided medical service to a confirmed case patient in Guangdong and developed cough and nasal congestion 2 days after exposed to the confirmed case patient. A triage nurse in Guangdong developed fever (37.8 ºC), cough and diarrhea 1 day after exposed to a confirmed case patient. Throat swabs collected from 28 ill contacts a median 1-day (range: 0-8) after their illness onset tested negative for H7N9 by rRT-PCR. Paired sera are being collected from contacts of cases for serological testing to assess H7N9 virus infections that might have been missed by virologic testing.

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