Supplementary Appendix

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

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Supplementary Appendices

Clinical Findings in 111 Cases of Influenza A (H7N9) Virus Infection

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Authors' Contributions

Drs. Lanjuan Li, Nashan Zhong, Chen wang (name in acknowledgement), Bin Cao and Hongzhou Lu designed the study. All the authors have been taking care of the H7N9 cases and have been involved in gathering data. Drs. Hainv Gao, Bin Cao, Bin Du, Qi Xia, Shi Gui Yang (name in acknowledgement) and Hui Li analyzed the data. Drs. Hainv Gao, Bin Cao, Qi Xia and Hui Li (name in acknowledgement) vouches for the data and the analysis. Dr. Bin Cao wrote the manuscript, Dr. Bin Du modified the manuscript. Drs. Bin Cao, Hainv Gao, Bin Du, Lanjuan Li and Nashan Zhong revised the manuscript. Drs. Lanjuan Li and Nashan Zhong decided to publish the paper.

Definitions of Moderate-to-severe acute respiratory distress syndrome (ARDS), rhabdomyolysis, pneumonia, acute kidney injury and exposure to live poultry

Moderate-to-severe acute respiratory distress syndrome (ARDS) was diagnosed according to ARDS Berlin definition, i.e. severe hypoxemia (PaO2/FiO2 ≤ 200 mmHg with PEEP ≥ 5 cmH2O), associated with bilateral opacities on chest X-ray, which could not be fully explained by cardiac failure or fluid overload. (1)

A patient was diagnosed as having rhabdomyolysis if medical record review showed that muscle pain or muscle weakness was present at the time of hospital admission and patient's creatine kinase level was more than 10 times the upper limit of normal.(2)

Pneumonia was diagnosed as an acute illness with cough and at least one of new focal chest signs, fever > 4 days or dyspnoea/tachypnoea, supported by chest radiograph findings of lung shadowing that is likely to be new and without other obvious cause.(3)

Acute kidney injury is defined as any of the following: increase in Serum creatinine (SCr) by ≥ 0.3 mg/dl (≥ 26.5 μmol/l) within 48 hours; or increase in SCr to≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or Urine volume < 0.5 ml/kg/h for 6 hours. (4)

The definition of exposure to live poultry was getting close contact with chicken or pigeons or visiting either a live poultry retailer or a market selling live poultry within two weeks before onset of illness. In this study, we excluded cases with "suspected" exposure (i.e. regular visit to the market without recalling the exposure date).

Case definitions

The case definitions for confirmed human infections with novel avian influenza A (H7N9) virus were based upon report from Qun Li and et al. (6) 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 real-time reverse transcription polymerase chain reaction (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.

H7N9 laboratory assays

Laboratory confirmation of H7N9 was same as those reported from Gao RB's report (5) and Qun Li' report (6). 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. The specific sequences have been published on the WHO website athttp://www.who.int/influenza/gisrs_laboratory/a_h7n9/en/. 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 hemagglutination 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 the confirmed case in Anhui. (5) 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.

Normal ranges for all laboratory chemistries and definitions for abnormal values

Normal range of White cells was 4,000-10,000 per cubic millimeter;

Normal range of Hemoglobulin for male was 12 -16 g/dl, for female was 11-15 g/dl;

Normal range of C-reactive protein was less than 10 mg/liter;

Normal range of Procalcitonin was less than 0.5 ng/ml;

Normal range of Aspartate aminotransferase was less than 40 U/liter;

Normal range of Creatinine was less than 133 umol/liter;

Normal range of Lactate dehydrogenase was less than 250 U/liter;

Normal range of Creatine kinase was less than 200 U/liter;

Normal range of Myoglobulin was less than 80 ug/ml;

Normal range of Potassium was 3.5 – 4.5 mmol/liter;

Normal range of Sodium was 135 – 145 mmol/liter;

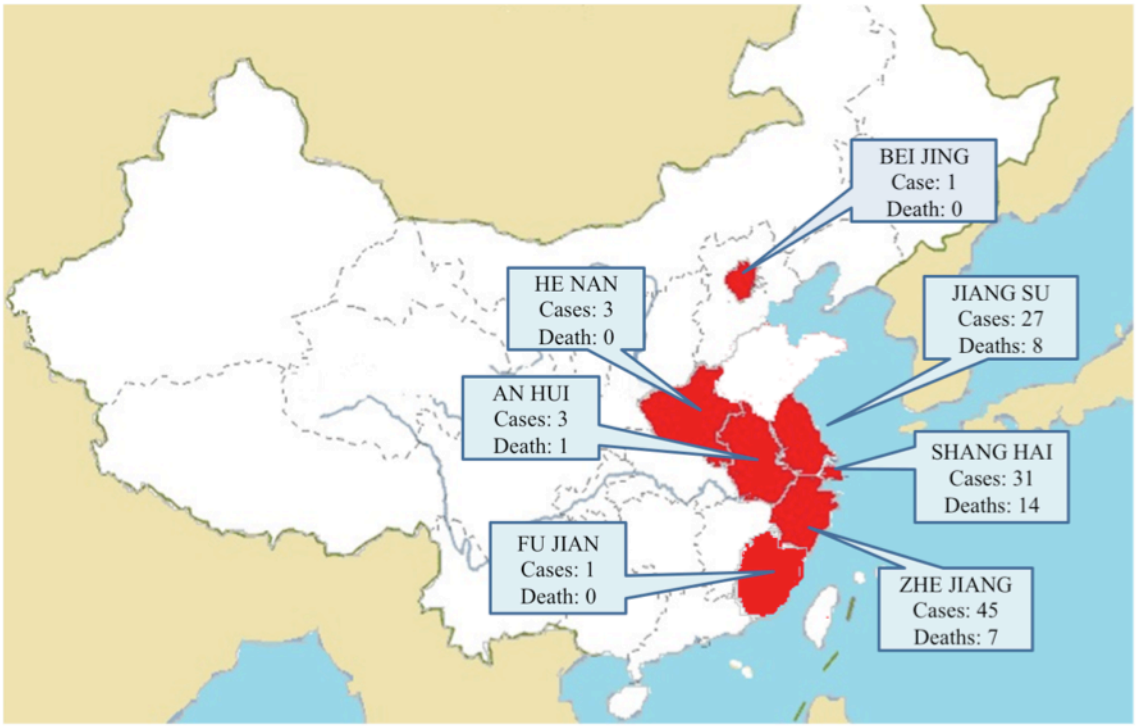
Normal range of D-dimer was less than 0.5 mg/liter;

Lymphocytopenia was defined as a lymphocyte count of less than 1500 per cubi millimeter;

Thrombocytopenia was defined as a platelet count of less than 150,000 per cubic millimeter;

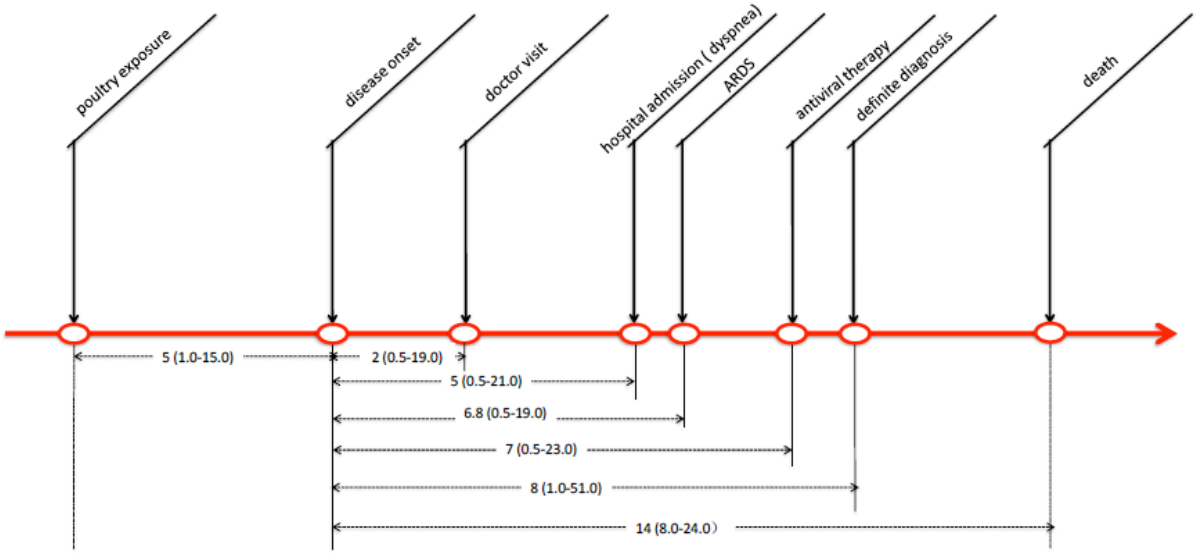
Abnormal value of PaO_2:FiO_2 was defined as the ratio of less than 300.

Figure S1 Distribution of confirmed H7N9 human cases in China included in our study



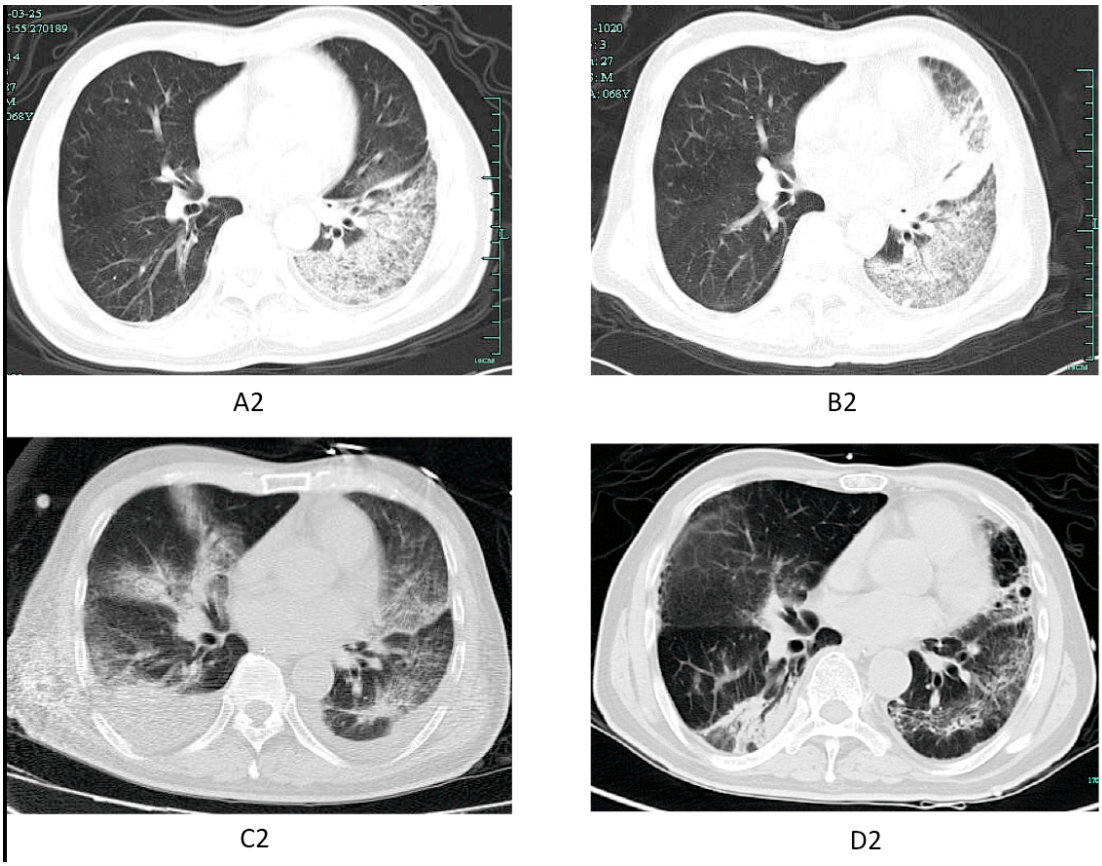
Distribution of 111 confirmed cases of human Infection with avian-origin H7N9 virus identified in China during the study period.

Figure S2 Natural history of human H7N9 cases



The median time interval from disease onset to poultry exposure and the median time intervals from doctor visit, the development of ARDS, hospital admission, antiviral therapy, lab-confirmed diagnosis, and death to disease onset were shown in this figure respectively. Numbers in parentheses indicate the ranges.The median time interval from dyspnea to disease onset was also 5 days ranging from 0.5 to 16 days.

Figure S3 Scans of the chest showing areas below the carina level in the patient with pneumonia caused by avian influenza A (H7N9) virus



A2: (Day 7) CT shows Unilateral Ground-glass opacities (GGO) and partial consolidation were observed on left upper lung on admission (Day 7).

B2: (Day 9) CT shows rapid progress of GGO and consolidation of left lung.

C2: (Day 16) CT shows increase of GGO of left lung and increase of consolidation of both lungs. Partial resolution of GGO is noticed of left lung.

D2: (Day 42) CT shows resolution of GGO and consolidation of both lungs with prodominant reticular changes.

Supplemental Table S1: Univariate analysis analysis of risk factors for ARDS of H7N9 human cases

Variables	*ARDS* (*n=79*)	*Without ARDS* (*n=32*)	*P value*
Sex (Male)	52 (65.8)	24 (75.0)	0.346
Age (⩾65ys)	39 (49.4)	8 (25.0)	0.019
Underlying medical condition	56 (70.9)	12 (37.5)	0.001
Current smoker	16 (20.3)	11 (34.4)	0.116
Sputum production	46 (59.0)	16 (50.0)	0.389
Hemoptysis	18 (22.8)	9 (28.1)	0.553
Diarrhea	7 (8.9)	3 (9.4)	1.000
Vomitting	3 (3.8)	2 (6.3)	0.953
Lymphocyte count＜1000/mm^3	68 (93.2)	23 (76.7)	0.018
Platelet＜10000/mm^3	35 (44.9)	9 (30.0)	0.159
AST＞40 u/L	58 (76.3)	15 (50.0)	0.008
CK＞200 u/L	40 (55.6)	9 (31.0)	0.026
LDH＞250 u/L	66 (91.7)	25 (86.2)	0.643
D-Dimer＞0.5 mg/L	32 (91.4)	15 (88.2)	1.000
Time from symptom onset to first visit >2 days	37 (46.8)	10 (31.3)	0.132
Time from symptom onset to admission > 6 days	33 (41.8)	11 (34.4)	0.512
Time from symptom onset to initiation antiviral therapy >3 days	72（91.1）	22（68.8）	0.004

Supplemental Table S2 Univariate analysis of predictor for death of H7N9 human cases, with those who had recovered from illness as control

Variables	*Death (n=30)*	*Recovery (n=49)*	*P value*
Sex (Male)	23 (76.7)	37 (75.5)	0.907
Age (⩾65ys)	18 (60.0)	17 (34.7)	0.028
Underlying medical condition	24 (80.0)	28 (57.1)	0.038
Current smoker	7 (23.3)	17 (34.7)	0.287
Sputum production	13 (44.8)	31 (63.3)	0.113
Hemoptysis	6 (20.0)	15 (30.6)	0.300
Diarrhea	4 (13.3)	6 (10.2)	0.952
Vomitting	1 (3.3)	2 (4.1)	1.000
Shortness of breath	21 (70.0)	23 (46.9)	0.045
Lymphocyte count＜1000/mm^3	25 (83.3)	41 (83.7)	0.304
Platelet＜10000/mm^3	15 (50.0)	18 (36.7)	0.222
AST＞40 u/L	24 (80.0)	30 (61.2)	0.060
CK＞200 u/L	15 (50.0)	19 (38.8)	0.157
LDH＞250 u/L	23 (76.7)	42 (85.7)	1.000
D-Dimer＞0.5 mg/L, n = 37	n = 9	n =28	
	7 (77.8)	26 (92.9)	0.515
ARDS	30 (100)	19 (38.8)	<0.001
Shock*	15 (50.0)	2 (4.1)	<0.001
Acute kidney injury	10 (33.3)	2 (4.1)	0.002
Time from symptom onset to first visit >2 days	16 (53.3)	17 (34.7)	0.103
Time from symptom onset to initiation of antiviral therapy >3 days	26（86.7）	40（81.6）	0.315
Time from symptom onset to initiation of antiviral therapy >5 days	21（70.0）	24（49.0）	0.024

*Shock at any point during the illness.

Supplemental Table S3 Multivariate logistic regression analysis of risk factors for death of H7N9 human cases

Factors*	Fatal cases (n=30)	
	Odds ratio (95%CI)	P value
Sex（Female)	1.04 (0.18-5.91)	1.00
Age≥65y	1.01 (1.00-1.04)	0.31
Underlying medical condition	0.63 (0.09-4.61)	0.65
Shortness of breath	0.56(0.10-3.21)	0.51
ARDS		1.00
Shock	6.51(1.09-38.92)	0.04
AKI	3.26 (0.46-22.85)	0.24
Time from symptom onset to initiation of antiviral therapy > 5days	1.93 (0.40-9.26)	0.41

* Reference group is male, age <65 years, without underlying medical condition, without shortness of breath, without ARDS, without shock, without AKI and initiation of antiviral therapy of 5 days or less.

Table S4 Selected laboratory abnormalities on admission of H7N9 human cases

Clinical characteristics and selected laboratory abnormalities	Value
Hemoglobulin - g/dl	
Median	12.9
Interquartile range	11.9 – 13.8
Total range	6.6 – 18.6
C-reactive protein - mg/liter	
Median	65
Interquartile range	25 – 113
Total range	1 – 330
Procalcitonin – ng/ml	
Median	0.4
Interquartile range	0.1 – 0.8
Total range	0.1 – 42.3
Aspartate aminotransferase – U/liter	
Median	53
Interquartile range	38 – 97
Total range	13 – 2814
Creatinine – umol/liter	
Median	71
Interquartile range	58 – 85
Total range	21 – 828
Lactate dehydrogenase – U/liter	
Median	498
Interquartile range	388 – 661
Total range	146 – 4471
Creatine kinase – U/liter	
Median	195
Interquartile range	96 – 562
Total range	24 – 4250
Myoglobulin – ug/ml	
Median	99
Interquartile range	45 –242
Total range	9 –1000
Potassium – mmol/liter	
Median	3.8
Interquartile range	3.4 – 4.1
Total range	2.6 – 5.4
Sodium – mmol/liter	
Median	136
Interquartile range	132 – 141
Total range	123 – 153

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