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LETTER

Human H9N2 Avian Influenza Infection: Epidemiological and Clinical Characterization of 16 Cases in China

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Dear Editor,

The first human infections with avian influenza virus (AIV) H9N2 were reported in 1998 (Guo *et al*. 1999). As of October 18th, 2019, 59 cases of human infection with H9N2 have been reported globally, including 50 cases in China, three in Bangladesh, four in Egypt, one in Pakistan and one in Oman (World Health Organization. https://www.who.int/wer/en/; Peacock *et al*. 2019). Among the 59 patients, three (5.1%) presented with severe pneumonia and 56 had mild influenza-like symptoms (World Health Organization. https://www.who.int/influenza/human_animal_interface/HAI_Risk_Assessment/en/). In general, human infections with H9N2 are sporadic and the majority of cases are mild and non-fatal, and there is no evidence of human-to-human transmission to date (Peacock *et al*. 2019).

During the 2018–2019 flu season, the number of patients with influenza-like illness that presented at Wuhan JinYinTan Hospital (Hubei, China) increased and more patients showing severe illness compared to previous years. To characterize the epidemic influenza virus, 54 oropharyngeal swabs collected from patients with influenza-like illness between January and February 2019, with influenza A-positive, were subjected to next-generation sequencing (NGS). Among the swabs, full-length genomes of H9N2 viruses were obtained from 16 swab samples, and the H9N2 isolates were confirmed by Haemagglutination inhibition (HI) assay and RT-PCR (see supplementary materials for details). Furthermore, these 16 orophyaryngeal swabs were negative for seasonal influenza (H1, H3, and influenza B virus) and other subtypes AIV tested by RT-PCR and the next generation sequencing. Namely, sixteen patients were confirmed to have H9N2 virus infection. 16 inpatients infected with H9N2 virus were included in this study. The history of hospitalization and physical examination, hematological, biochemical, radiological, and microbiological test results were collected. Among 16 patients, 11 (68.8%) had severe illness (including four deaths), whereas symptoms of H9N2 infection are usually mild. We further explored the epidemiological and clinical characteristics of these cases to provide a much-needed theoretical basis for the prevention and treatment of human H9N2 infection.

The median age of the 16 patients was 61.5 years old (range 13 months to 88 years old) (Supplementary Table S1). Five patients (31.3%) were 65 years of age or older, two patients (12.5%) were infants. There were more male (n = 10) than female patients (Supplementary Table S1). The 16 confirmed cases were distributed over five districts of Hubei Province, and 56.3% (9/16) were in Wuhan, the capital city of Hubei Province (Supplementary Figure S1).

To identify the sources and transmission routes of the human H9N2 AIVs, an epidemiological retrospective study was conducted in seven patients between June and July 2019 (other nine patients were failed to be contacted) (Table 1). Five (71.4%) patients had antibodies against H9N2 in their convalescent sera as indicated by HI assay (Table 1, Supplementary Table S2). Among the seven patients (median age, 61 years old), five (71.4%) were male. Most patients

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Table 1 Demographic and epidemiological characteristics of seven patients with confirmed H9N2 virus infection in Hubei Province, China.

Characteristic	Value
Age (years)	
Median (range)	61 (48–69)
Subgroup, no. (%)	
0–4	0
5–14	0
15–49	2 (28.6)
50–64	4 (57.1)
≥ 65	1 (14.3)
Male, no. (%)	5 (71.4)
Occupation, no. (%)	
Peasant	1 (14.3)
Self-employed	2 (28.6)
Retired	4 (57.1)
Underlying health conditions, no. (%)	5 (71.4)
Exposure to poultry, no. (%)	3 (42.9)
Direct contact with chickens and ducks	1 (14.3)
Direct contact with Muscovy ducks	1 (14.3)
Visit to live poultry markets	1 (14.3)
Travel history, no. (%)	0
Serologic test (anti-AIV H9N2-positive), no. (%)	
Patients	5 (71.4)
Family members of patients	6 (85.7)
Patients' close contact with flu symptoms	3

(5/7, 71.4%) had underlying health conditions. Three patients (patients 7, 8, and 10) had been exposed to live poultry. None of the patients had ever traveled (Table 1). Thirteen serum samples were collected from six patients (patient 4 died) and seven family members. Six out of seven tested family members carried anti-H9N2 antibodies (Table 1, Supplementary Table S2), none of them had a history of poultry exposure. Three family members of two patients had also developed flu-like symptoms, and two of them carried H9N2 antiboby (wife of patient 1, patient 1-W; husband of patient 16, patient 16-H; the sera of father of patient 16 was not collected). These findings indicate that, there is a possibility of human-to-human transmission of H9N2.

Among the 11 patients, five had mild illness, 11 had severe illness, and four of the 11 patients died from the disease (Supplementary Figure S2). Five patients (two males, two infants; median age, 48 years old) presented with mild symptoms, including fever, cough, expectoration, chest distress, or vomiting. Lab tests and chest X-ray consistently revealed that the patients had a viral infection. Five patients had no underlying health conditions and received oseltamivir within 48 h after admission. All five mild cases fully recovered, with neither sequelae nor relapse (Supplementary Table S3, Supplementary Table S4, Supplementary Figure S2).

Of the 11 patients with severe disease, eight were male, and the median age was 62 years old (range 46–88 years old). The majority (81.8%, 9/11) of the patients had underlying health conditions, such as hypertension, diabetes, chronic obstructive pulmonary disease, or gastric ulcer. Five patients had cardiovascular diseases, two had respiratory diseases, and two had endocrine system diseases, and one patient (patient 5) even had four chronic diseases (Supplementary Table S5, Supplementary Table S6). In the early disease stage, all 11 patients presented with cough, expectoration, chest distress, and dyspnea. In the late stage, a decrease in lymphocytes and an increase in neutrophils were seen in all 11 cases (100%). Nine patients presented increased procalcitonin. Extensive lung lesions and respiratory failure were prominent symptoms in all 11 severe cases, and sepsis and acute respiratory distress syndrome (ARDS) were the major complications. For some of the patients, bacteria and fungi were detected in routine sputum cultures (Supplementary Table S5, Supplementary Table S6).

All 11 patients with severe disease underwent chest radiographs on admission, and chest CTs revealed typical features of pulmonary infection, with ground-glass opacity and consolidation. CT pictures of one 62-year-old patient (patient 7) were used to present the progress of viral pneumonia and the gradual improvement after treatment. On day 13 after disease onset (Fig. 1A), widespread ground-glass opacity, and consolidation were seen bilaterally. The patient was given antiviral, antibiotic, and symptomatic treatment that led to a decrease in bilateral ground-glass opacities on day 21 (Fig. 1B). Since then, the patient mainly received symptomatic treatment, and stopped using antiviral and anti-infective drugs. On day 28, the patient was discharged from the hospital as his symptoms had improved. When he came back to the hospital for a check on day 53 (Fig. 1C), the opacities had completely disappeared.

All 11 patients with severe illness were treated with antiviral medication, systemic antibiotic therapy, and other supportive treatment. Four patients eventually died, seven improved to full recovery (Supplementary Table S5, Supplementary Table S6). In the fatal cases, the median time from symptom onset to respiratory failure was 14 days (Supplementary Figure S2). The patients showed dyspnea and hypoxemia upon being transferred to the intensive care unit (ICU) and developed complications, such as ARDS and sepsis. Patient 2 was diagnosed as having multiple bacterial infections and died of severe pneumonia and sepsis. Patient 4 had no underlying blood system disease, but died of severe anemia and respiratory failure due to autoimmune hemolytic anemia. Patient 6, an 84-year-old male with a history of immune disorder, died of pulmonary infection because of refusing treatment. Patient 9 developed hypoxemia, but refused mechanical ventilation and died of respiratory failure.

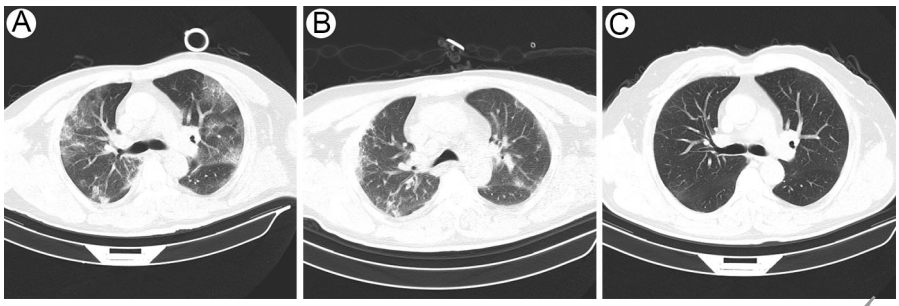

Fig. 1 Chest CT of patient 7, a 61-year-old male with severe pneumonia caused by AIV H9N2. **A** On day 13 after disease onset, widespread ground-glass opacity, and consolidation were seen bilaterally. **B** On day 21, there was a mi[illegible]focal ab[illegible]orption. **C** On day 53, the shadows had completely disappe[illegible]

Diagnostic indicators of infection (percent neutrophils, procalcitonin) and heart failure (natropeptide precursor) were significantly increased in the four patients (Supplementary Table S5 and Supplementary Table S6).

All patients with severe illness had lower respiratory infection symptoms (especially dyspnea) at the onset of illness, which rapidly deteriorated into severe pneumonia, ARDS, and/or sepsis, and four patients even died. These findings are quite different from those in human H9N2 infection cases reported previously (Gu *et al*. 2017), but they are similar to those reported in human infections with AIV H7N9 (Chen *et al*. 2013; Gao *et al*. 2013a, b). Notably, for the four patients who deceased, there was a relatively long period between initial symptom and clinical deterioration because the patients were diagnosed as having bacterial pneumonia or interstitial pneumonia in local hospitals, without consideration of a virus infection. The patients' conditions began to deteriorate three to four days after disease onset, with the development of dyspnea and hypoxemia, which was a turning point, as then, the patients rapidly developed ARDS, multiple organ failure, and other fatal complications. Thus, timely antiviral therapy, correction of hypoxemia, and timely control of complications are crucial to increase the cure rate of H9N2 infection. Our findings suggest that H9N2 infection can lead to ARDS and sepsis and eventually resulting in multiple organ failure and death. It is worth noting that the 11 patients with severe disease in this study, especially the deceased patients, had a history of underlying health conditions, such as hematologic disease, respiratory disease, or immune dysfunction, which rendered patients more susceptible.

The independence test between the clinical characteristics and severe/mild cases and death in severe cases were done by using Fisher's exact test. A P value of less than 0.05 was considered statistically significant. Univariate analysis showed that the risk of human H9N2 severe case was increased among patients who had an underlying health condition, among those who had a dyspea or chest stress at the onset of illness, and among those who developed into ARDS or sepsis during the illness (Supplementary Table S7). Univariate analysis also showed that the predictor for death of H9N2 human severe case was increased among patients who deteriorated into acute renal injury (Supplementary Table S8). However, we failed to do multivariate analysis of risk factor to identify independent predictors of human H9N2 mild to severe cases and/or severe cases to death because of too small size of samples.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement The study was conducted in accordance with the Declaration of Helsinki and with the ethical standards of the responsible committee on human experimentation. This study was approved by the institutional review board of Wuhan JinYinTan Hospital (reference number: KY-GR-2019-02.01). All patients provided signed informed consent, and any patient information, including illustrations, has been anonymized.

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