

## Case Report

# A case of human infection with avian Influenza A/H7N9 virus in Beijing: virological and serological analysis

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### Abstract

This study described some essential viral and sera-characteristics of A/H7N9 virus infection in a 61-year old case patient. With the antiviral therapy and respiratory support, the patient showed a significant decrease of viral loads from 6.72 log<sub>10</sub> copies/mL to 0 in 22 days, and a correlated increase of serum hemagglutination inhibition titers during the same period. Phylogenetic analysis demonstrated that the isolated strain was highly homologous to previous strains isolated in the southeast of China. Drug resistance-associated R292K mutation became detectable 17 days after antiviral therapy, but no remarkable influence on the viral clearance was observed.

**Key words:** Influenza; H7N9 avian influenza; drug resistance; antibody; viral load.

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### Introduction

In February 2013, the first case of human infection with a novel avian-origin reassortant influenza A/H7N9 was reported in Shanghai, China [1]. As of Oct. 2, 2014, a total of 453 human H7N9 virus infections with 165 fatal cases were reported [2]. It attracted much attention due to viral variations, rapid progression and potential human-to-human transmission of the H7N9 infection [3-5]. Thus a number of studies have been published to investigate the general information on clinical features, gene reassortment, drug resistance, among others, but detailed case studies focused on viral characteristics are still scarce. Therefore in this study, we described a dynamic monitoring of the viral loads, serological responses and drug-resistance R292K mutation in a confirmed H7N9-infected case patient. Our data is of value as reveal the viral characteristics in H7N9 infection and support the evaluation of treatment strategies.

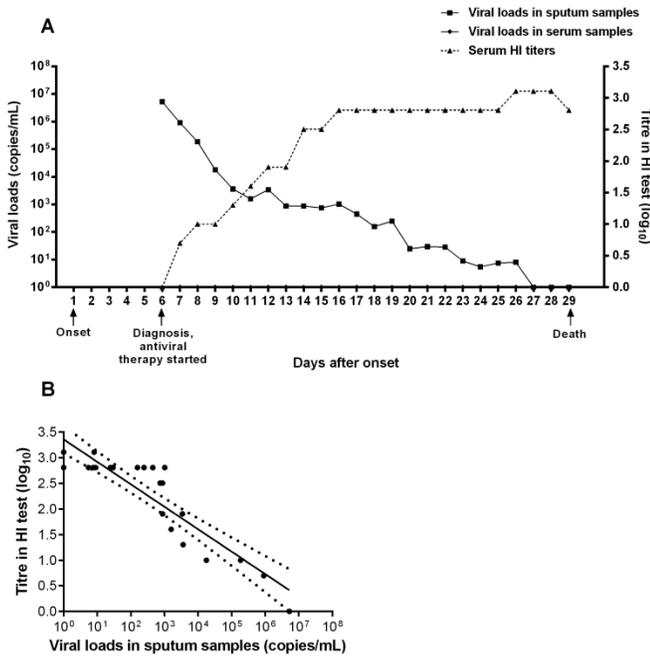
### Methodology

A 61-year old woman was admitted to the Beijing ChaoYang Hospital with a history of five 5 days fever without apparent causes. This case was confirmed as H7N9 infection on July 19, 2013. Although oseltamivir capsules, intravenous peramivir, veno-

venous extracorporeal membrane oxygenation (VV-ECMO) and continuous veno-venous hemofiltration were administered on the same day of the lab diagnostic results, the patient developed a blood stream infection by a multidrug-resistant *Acinetobacter baumannii*, which led to septic shock and death of the patient on August 11, 2013.

To confirm the infection and administer the subsequent treatment, H7N9 viral loads in sputum and serum were measured. Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. Quantitative real time polymerase chain reaction (RT-PCR) was performed with a one-step Taqman real-time RT-PCR kit (Qiagen) using the protocol of the World Health Organization Collaborating Center in Beijing [6]. The H7N9 viral loads were quantified using a standard plasmid which contained H7N9 matrix gene (synthesized by Sangon Biotech, Shanghai, China), and showed as log<sub>10</sub> cDNA copies per mL (Figure 1A). The viral load in the first sputum sample collected 6 days after the onset of clinical symptoms was 6.72 log<sub>10</sub> copies/mL. As a result of the antiviral therapy and respiratory support, the viral loads in sputum samples decreased, from 6.72 log<sub>10</sub> copies/mL on day 6 to 0.87 log<sub>10</sub> copies/mL on day 25. After day 29, no H7N9 viral RNA could be detected.

**Figure 1.** Profile of H7N9 viral loads and serum HI titers in the case patient A, the profile of H7N9 viral loads and serum HI titers in case patient; B, the correlationship between viral loads and serum HI titers in the case patient ( $R^2 = 0.824$ ,  $P < 0.001$ ). 95% CI (-0.5272 - (-0.3486)) was shown by dotted line.

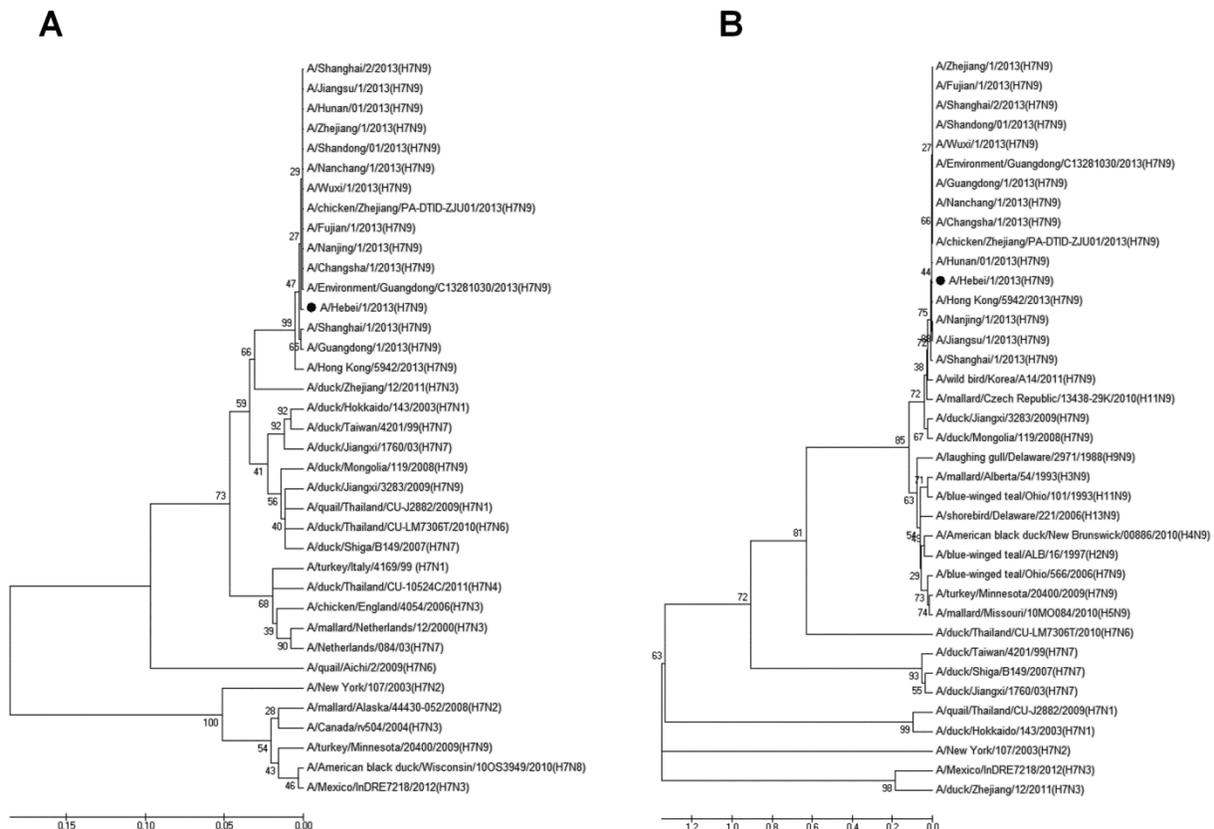


The mean of viral loads in acute-phase samples (collected less than 7 days after symptom onset) was 6.34  $\log_{10}$  copies/mL, while the mean value registered during the convalescent-phase >14 days after symptom onset was 1.74  $\log_{10}$  copies/mL. Notably, no H7N9 viral RNA was detected in any serum samples.

The case patient H7N9-specific antibody titers were then determined. Hemagglutination inhibition assays (HI) were performed by using the inactivated H7N9 vaccine strain NIBRG-268 (Sinovac, Beijing, China) and 1% horse blood cells according to the protocol developed by the Chinese Center for Disease Control and Prevention (CDC) [7]. It was shown that HI titers increased continually during the first 11 days after diagnosis (16 days after onset). Moreover HI titers in acute-phase serum ranged from 0 to 0.70  $\log_{10}$ , and from 2.51  $\log_{10}$  to 3.11  $\log_{10}$  (GMT 2.84  $\log_{10}$ ) for serum collected at convalescent-phase. The linear regression analysis showed that viral loads were correlated with the HI titers ( $p < 0.001$ , 95% CI: -0.5272 - [-0.3486],  $R^2 = 0.824$ ) (Figure 1B).

Since neuraminidase inhibitors (oseltamivir capsules and intravenous peramivir) were applied immediately after the diagnosis, we continually monitored the viral variations of Arg292Lys (position

**Figure 2.** The phylogenetic tree of HA and NA genes; A, the phylogenetic tree of HA gene; B, the phylogenetic tree of NA gene. The dots indicate the case strain.



292 with N2 numbering; position 294 with N9 numbering), which might lead to the drug-resistance. The mutation assay was performed using the Taqman real-time RT-PCR previously described [8], and the RNaseP was chosen as an internal control. The relative expression level of mutated viruses was calculated with the  $2^{-\Delta Ct}$  method. It was noteworthy that a mixed population of arginine and lysine at position 292 from the neuraminidase (NA) gene was detected from samples collected on day 22 (17 days after the antiviral therapy), 23 and 26. Percentages of drug-resistant viruses in these samples were 0.19%, 0.10%, and 0.39%, respectively.

H7N9 viral genome was amplified using influenza A conserved primers [9], and then determined by Sanger sequencing (Sangon Biotech, Shanghai, China). Phylogenetic analysis was performed for hemagglutinin and neuraminidase gene of tested samples, of 36 other H7-strains and 37 other N9-strains, respectively. The lowest evolutionary distance of the HA gene was 0.0031 (SD 0.0028), appeared between the tested strain and A/Zhejiang/1/2013(H7N9), while that of the NA gene was 0.0093 (SD 0.0032), occurred between the tested strain and A/Hunan/1/2013(H7N9) (Figure 2). Based on the genetic heterogeneity, our tested strain belonged to the genotype G0, which was highly consistent with previous isolates from southeastern China [4].

## Discussion

The H7N9-infected patient reported here had a severe case with rapid progression to a bloodstream infection with multidrug-resistant *Acinetobacter baumannii* and multiple organ failure (MOFE); the patient died 29 days after the onset of respiratory symptoms. Considering the clinical characters of this typical H7N9-infected patient that have been reported [10], we focused on the viral features, performing a continuous monitoring, and made some notable findings. First, a good response to the antiviral treatment was observed. With the immediate peramivir/oseltamivir therapy initiated after the diagnosis of the H7N9 infection, viral loads decreased gradually and showed a negative conversion on day 27. Notably, no viral RNA could be detected in the serum specimens during the whole process, which did not fully corresponded with previous studies. Although Lin P *et al.* also did not detect the viral RNA in a severe case [11], Hu Y *et al.* reported that twelve out of fourteen H7N9-infected cases (86%) were positive in serum viral load test [8]. Based on the

difference of admission, we believe that the viremia should be highly relevant with immunosuppression, which was mainly caused by the application of corticosteroid in Hu Y *et al.*'s study. Second, we confirmed the significant negative correlation between viral loads and serum HI titers in this study. Because the correlation between titers of HI and neutralizing antibodies (NAbs) has been proved [12], the titers of HI should be treated as a potential marker to evaluate the viral clearance in H7N9-infected cases.

It has been known that the use of neuraminidase inhibitors and delayed viral clearance may cause the emergence of resistance, such R292K in viral NA gene [1,8]. However, our case patient, with continual peramivir treatment and a positive window of H7N9 viral RNA lasted for 26 days, showed no R292K mutation until the last few days. Hu Y *et al.* reported an ECMO-supported patient, who had 100% NA Arg292 at the beginning, while showed 100% Lys292 7 days after treatment [8]. Compared with the latter, the patient described in our study received a similar treatment (ECMO and Peramivir/Oseltamivir), but the emergence of R292K mutation was much slower (7 days vs. 17 days) and non-significant (100% vs. < 0.4%). Due to both low viral loads and low ratios of the 292K genotype in the samples collected on day 22, 23 and 26, the virus isolation and the determination of the fifty percent inhibitory concentration ( $IC_{50}$ ) of neuraminidase inhibitors could not be achieved. Our data clearly indicated that the minor R292K mutation may not have great influence on the viral clearance, if the H7N9 virus replication has been well controlled.

## Conclusion

In conclusion, the case patient described in this report exhibited a significant decrease of viral loads under the influence of the antiviral therapy and of the H7N9-specific immune responses. Peramivir resistance-associated R292K mutation appeared in the last stage of the disease process, but showed no remarkable influence on the viral clearance. Combining our studies with the previous clinical report [10], a most detailed case analysis was achieved, which is of great value to get a deeper insight of the H7N9 infection.

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**Conflict of interests:** No conflict of interests is declared.