1 **Title page**

2 **High thermostability of the newly emerged influenza A**
3 **(H7N9) virus**

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13 **Running title:** High thermostability of H7N9 viruses

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Dear Editor ,

To and colleagues (1) described unique reassortant H7N9 viruses associated with severe disease in Hong Kong, that has aroused global fear of a new pandemic. Since its emergence in March, 2013, in China, this novel H7N9 virus has caused more than 500 laboratory-confirmed cases with over 30% fatality. Bioinformational analysis showed that this deadly human invader originated from multiple reassortments between circulating H7/N9 and H9N2 avian influenza viruses (2). The biological features of this virus have been well characterized by different investigators, and critical amino acid mutations that facilitate transmission from human to mammals are identified (3, 4).

Influenza virus transmission is a complex process involving virus infectivity, persistence in environment and infection of next host. Thermostability has been well recognized as an intrinsic characteristic related to infectivity persistence; higher thermostability results in longer duration thus more efficient transmission (5). The seasonality of influenza epidemics is highly synchronized with temperature variation yearly, indicating temperature may affect virus transmission(6). However, the thermalstability of the newly emerged H7N9 virus and its biological importance for its emergence remains largely unknown.

Herein, we compared the thermostability of H7N9 virus strain A/Anhui/1/2013(AH1) with the 2009 pandemic H1N1 strain A/California/07/2009(CA07) and representative epidemic seasonal H1N1 strains A/Beijing/HZ01/2013(HZ01) and A/Porto Rico/8/34(PR8). Avian influenza virus H9N2 strain A/chicken/Nanjing/1/2013(NJ1) circulating in poultry in southern China(7), which provided the six internal genes for the H7N9 virus, was also included for comparison. All viruses were prepared with 9-day-old embryonated chicken eggs and titrated on MDCK cells by standard plaque assay. Each virus aliquot was incubated at 50 °C for various times, after which the loss of infectivity were determined by plaque assay on MDCK as previously described (8). All experiments

involving infectious viruses were performed in Biosafety Level-3 facilities at Beijing Institute of Microbiology and Epidemiology, China.

As shown in Fig 1, these viruses could be divided into two distinct clusters: AH1 and CA07 viruses, characterized with higher thermostability, while epidemic strains, HZ01 and PR8, were sensitive to heat ($P < 0.005$). CA07 (pH1N1) and AH1 (H7N9) are very similar (-0.0222 ± 0.00213 and $-0.0259 \pm 0.00115 \log_{10} \text{PFU/ml min}^{-1}$) in loss of infectivity upon heat treatment; both viruses are deemed as unexpected incomers for human, and their high thermostability probably favored their first jump from swine or avian to human being. A preliminary virus inactivation test also confirmed the H7N9 virus could tolerate high temperature(9). Especially, AH1 was more heat-resistant compared with its parental H9N2 strain NJ1, indicating the close relation between viral surface glycoprotein and thermostability. Recombinant H5/H1 hybrid virus carrying a single mutation in HA was recently evidenced to enhance thermostability and transmit more efficiently among mammals (8). Recently, mutational analyses based on H9N2 virus showed that HA and PA contained critical determinants for airborne transmissibility among chickens (10). However, the molecular mechanism behind this deserves extensive investigation in the future.

Our results highlight the importance of high thermostability trait of H7N9 viruses to the emergence and transmissibility in human. The multiple reassortment offers H7N9 viruses long duration and survival in live poultry market and increases opportunities of human contact. We call for extensive surveillance and thermostability characterization of newly recognized viruses to help figure out the transmission and pandemic potential in human. Further gain-of-function study will clarify the correlation between thermostability and transmission determinants.

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130 **Figure Legend**

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132 Figure 1 Thermostability of representative influenza A viruses. All viruses were
 133 prepared with 9-day-old embryonated chicken eggs and titrated on MDCK cells by
 134 plaque assay. Virus titers decreased with time, and rates were determined with unit
 135 log₁₀ PFU/ml min⁻¹. Linear regression was used to obtain best-fit lines. Slopes (±
 136 standard errors) are indicated, and asterisks denote P<0.005 for significant difference.

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