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Mammalian adaptation markers in avian-origin H7N9 virus, a comprehensive investigation in isolates and clinical specimens from the H7N9 influenza affected area

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Background: An avian-origin H7N9 virus emerged in eastern China in February 2013 and has since caused 133 confirmed human infections (Gao R, et al. *N Engl J Med*. 2013;368:1888-1897). We have compared human isolates and avian viruses isolated from epidemiologically linked poultry markets and confirmed that the human infections were caused by direct transmission from a poultry source (Chen Y, et al. *Lancet*. 2013;381:1916-1985). In addition to the Q226L substitution in the HA, which may provide virus with some ability to bind to human-type receptors, what other adaptations has this virus gained to make it different from other avian influenza viruses? Materials and Methods: We characterized H7N9 and other related H7 and N9 subtype viruses isolated in April 2013 from local poultry markets that were associated with human infections. Genetic mutations, polymerases activity, growth kinetic in mammalian and avian cells and replication ability in mice were determined using reverse genetic versions of H7N9 virus. Results: Replication ability and growth kinetics of the avian H7 subtype influenza viruses were compared in avian and mammalian cell lines. Our data suggest that the reassortant H7N9 virus has adapted to, and may have become established in, land-based poultry. It is currently not clear if this virus may still be circulating in some poultry populations, continuing to evolve and posing a threat for further human infections. We studied the virus genome for mammalian adaptation markers by performing sequence analysis on virus isolates and RT-PCR products derived from samples obtained from 46 patients hospitalized in the First Affiliated Hospital of Zhejiang University Medical School, Hangzhou, China. Virus adaptation markers in the HA, NA and PB2 genes were analyzed in sequential samples. Multiple adaptation markers were identified in these genes of clinical isolates and serial respiratory samples. Our data showed that the avian-origin H7N9 has attempted to adapt to replicate in human cells using various mechanisms already displayed by other viruses. An in vitro assay showed that viruses with substitutions at these positions exhibit enhanced RNP polymerase activity, and a study of growth kinetics demonstrated that isolates carrying these adaptation markers replicate to a higher titer in mammalian cells. Replication efficiency of these clinical variants was also evaluated in mice. As neuraminidase inhibitors are used as the first line of antiviral drugs for the treatment of H7N9 infection, we also analyzed oseltamivir resistance-associated mutations in the NA genes of viruses shed in either serial nasal swab or sputum specimens obtained from 40 of the hospitalized patients. Conclusions: This study provides a comprehensive analysis of avian-origin H7N9 virus from poultry and in human infections. The novel H7N9 virus attempted multiple adaptive strategies for efficient replication in humans. Further characterization of this H7N9 avian influenza virus for understanding the mechanism of replication adaptation and the role in efficient human transmission is necessary.

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