Molecular evolution of H9N2 influenza A viruses in Eastern China (1996 to 2008): implications for the origin of highly pathogenic H5N1 viruses

Pinghu Zhang1,2, Xufan Liu1, Yinghua Tang1, Xiaowen Liu1, Wenbo Liu1, Xiaorong Zhang1, Hongqi Liu1, Daxin Peng1, Song Gao1, Xiantao Wu1, Shan Lu3,4. 1Animal Infectious Disease Laboratory, College of Veterinary Medicine, Yangzhou University; 2New Drug Screening Center, China Pharmaceutical University; 3The First Affiliated Hospital of Nanjing Medical University; 4University of Massachusetts Medical School, Worcester, MA, USA

Background: Since it was first isolated in Southern China in 1992, H9N2 avian influenza virus has caused significant economic loss in China and is transmitted to humans and other animals. Currently, H5N1 avian influenza virus have resulted in 285 deaths of 364 diagnosed humans cases. However, the precursors of these H5N1 influenza viruses are yet to be determined.

Methods: We analyzed the antigenic and genetic features of H9N2 influenza viruses isolated from poultry in Eastern China from 1996 to 2008.

Results: Phylogenetic analysis revealed 6 genotypes, including 4 novel genotypes that have not been recognized before. The major H9N2 influenza viruses represented by A/Chiken/Beijing/1/1999 (Ck/BJ/1/99)-like viruses circulating in poultry in eastern China before 1998 were gradually replaced by A/Chicken/Shanghai/F/1998 (Ck/Sh/SH/F/98)-like viruses, which contained RNP complex genes genetically highly related to H5N1 influenza virus, since 1998. The similarity of the RNP genes in Ck/SH/F/98-like viruses to those of the human and poultry H5N1 influenza viruses isolated from 2001 onwards circulating in Eurasia suggests that the Ck/SH/F/98 virus may have been the donor of internal genes of these H5N1 viruses.

Conclusion: Our study provides new insights into the genesis and evolution of H9N2 influenza viruses and provides new evidence for the origin of H5N1 influenza viruses.

OL-045 The establishment of real-time fluorescent quantitative polymerase chain reaction (PCR) for detection of highly pathogenic Avian influenza virus subtype H5N1

Changran Zhang1,2, Yuanyuan Niu1, Xiaoyuan Liu1, Kaixuan Zhou2, Jiongcong Lin1, Ming Li1, Xiaohua Luo2. 1Department of Internal Medicine, Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University; 2The Huang Pu Center of Disease Control and Prevention (CDC)

Background: Highly pathogenic strains of avian influenza virus (AIV), which are influenza A viruses, cause severe disease in domestic poultry and humans. The objective of this study was to establish a fluorescent quantitative RT-PCR assay for detection of highly pathogenic avian influenza virus (AIV) subtype H5N1.

Methods: The H5 and N1 subtype-specific probe sets were developed based on avian influenza virus sequences detected in China. Two pairs of primers and two fluorescent probes were strictly designed and optimized in a reaction system. According to the amount of plasmid RNA extracted from H5N1 strains, the standard curve DWQBGWQDWQBGW of fluorescent quantitative PCR was drawn and all of the specimens were then tested by means of Real-time PCR.

Results: The standard avian influenza A virus/H5N1 panel appeared positive (10/10, 100%), which demonstrated by virus isolation (VI). H9N2 and H3N2 were all negative (10/10, 100%); Newcastle disease virus (NDV), Infectious bronchitis virus (IBV), Infectious bursal disease virus (IBDV), egg drop syndrome virus (EDSV), and the blank control were all negative (10/10, 100%). 11 sick or dying chickens, which were diagnosed AIV, appeared positive (10/11, 90.9%); 65 staffs' throat swab samples also were collected who worked as sellers of chickens in the bird markets were all negative (0/65, 0%). The assay was easy to carry out and highly reproducible.

Conclusion: Fluorescent quantitative PCR, described here, provides a rapid, specific and sensitive method to detect not only the H5 but N1 genes as well, in particular, during outbreaks of H5N1 influenza A virus.

OL-046 Comparative study of rapid test with standard method RT-PCR for detection of influenza A virus infection in suspected cases admitted at Khon Kaen Hospital, Thailand during 2005–2007

Pairoj Wechagama*, Wisansanee Thaewpia, Rinjong Promsorn, Sompong Jintanongthai. Clinical Pathology Laboratory Department, Khon Kaen Hospital, Khon Kaen, Thailand

Objectives: To assess the performance and effectiveness of rapid test; Clearview Exact Influenza A&B (Binax, UK) for detection of influenza A virus in nasopharyngeal swab by using RT-PCR as the standard method.

Method: Patients with a influenza-like illness admitted at Khon Kaen Hospital between January 1, 2005, and October 31, 2007, were considered for this study. Samples were collected with informed consent. In total, 50 suspected cases with nasopharyngeal swab samples were available for the study. The clinical benefit of the rapid test was evaluated by comparing the results with standard method RT-PCR.

Result: The prevalences of influenza A positive in 50 cases measured by rapid test and RT-PCR are 23.8% and 66.7%. The sensitivity, specificity, positive predictive value, negative predictive value and prevalence are 83.33% (95%CI 51.5-91.9,), 100% (95%CI 88.4-100), 100% (95%CI 69.1-100), 93.75% (95%CI 79.2-99.2) and 28.57% (95%CI 15.7-44.6) respectively.

Conclusion: The finding that the sensitivity of rapid test was slightly low when compared with standard method RT-PCR. There may depend on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities. In addition, the important properties of diagnostic tests that need to considered are analytical sensitivity and analytical specificity.

OL-047 A combinatorial antiviral approach against influenza A virus using ribozyme and siRNA

Madhu Khanna1,2, Vikas Sood1, Akhil Banerjea3, Prashant Kumar3. 1VP Chest Institute; 2University of Delhi; 3National Institute of Immunology, New Delhi

Background: Recent advances in antisense technology have emerged as a ray of hope against many pathogens. Latest fed