virus was first identified in March 2013, three seasonal epidemic waves have been detected in China. Live poultry market (LPMs) exposure is regarded as a major risk of H7N9 virus infection. However, despite strict interventions implemented in the epicenter cities during each outbreak, reports indicate a gradual nationwide spread of the virus. In this study, the impact of LPMs interventions in virus persistence and transmission in the province of Guangdong, the epicenter of the second and third epidemic waves, was assessed by genetic and spatial analyses. We analyzed the temporal and spatial distribution of all of H7N9 clinical cases reported in Guangdong from August 2013 to March 2015. Viral isolation and genome sequencing were performed for 81 of all 182 clinical H7N9 infection cases as well as for 65 H9N2 viruses from live poultry and LPMs environments, from 16 prefecture-level cities during the three epidemic waves. Molecular clock and spatial phylogenetic analyses were applied to trace virus persistence and transmission across epidemic waves, genetic segments and geographic regions. Temporary LPMs closure in epicenter cities reduced by 35% the number of clinical cases from 110 in the second wave to 72 during the third wave. However, eastern Guangdong, which reported few cases of H7N9 infection in the second wave, became the new epicenter of H7N9 outbreak during the third wave. Genetic analyses of the virus external genes showed, with strong support, that the third wave outbreaks in central and eastern Guangdong are the result of virus persistence rather than virus importation from elsewhere. Analyses of the internal genes of H7N9 virus from the third wave sampled in Guangdong indicate additional reassortment events with virus lineages from central and eastern China. Guangdong province reported the highest number of H7N9 cases during the second epidemic wave. In response to the outbreak, LPMs closure in epicenter Guangdong cities has been implemented. However, our study shows that the LPM closure in epicenter Guangdong cities during the second wave was insufficient. We find that the viruses responsible for the third wave outbreak in Guangdong descend from the virus circulating in the second wave in the same region. In addition, the newly identified reassortment events between Guangdong H7N9 internal genes and those from strains circulating in other provinces suggest that LPMs closure without a ban of poultry trade may contribute to the increase of H7N9 virus genetic diversity through reassortment with imported strains. Importantly, that a new outbreak in eastern Guangdong during the third wave was caused by a rarely detected virus that was circulating in the same region during the second wave, raising an alarm that the cities outside of H7N9 outbreak epicenters should not be neglected from the list of LPM interventions.

A7 Evolution of influenza A(H7N9) viruses from waves I to IV

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The H7N9 influenza virus that emerged in East China in early 2013 has caused 736 human infections with a fatality rate of 38.5%, through four outbreak waves. Our previous studies revealed that this virus was generated by reassortment between viruses from wild bird H7 and N9 viruses (surface genes) and poultry H9N2 viruses (internal genes), and that while the H7N9 wave I viruses had highly similar surface genes, the surface genes of the wave II viruses developed into regionally distinct clades. The H7N9 viruses continued to reassort with different H9N2 viruses to obtain internal gene segments, thereby generating multiple variants or genotypes. Our ongoing surveillance suggests that the H7N9 virus has become enzootic in chickens, and disseminated to most regions of China during waves III and IV of the outbreak. In this study, we have generated more than 800 H7N9 virus full genome sequences, and are analyzing these together with all genomes available in public databases. We are exploring the following scientific questions: (i) what is the continuing evolutionary behavior of the H7N9 virus lineage; (ii) what are the interactions or gene transfers between circulating H7N9 viruses and other enzootic influenza viruses, and the changes in genotypes over the four waves; (iii) what are the interactions among sub-lineages or clades, i.e. predominance and/or sub-lineage replacement; and (iv) what is the development and dissemination of the H7N9 viruses from a phylogeographic perspective. We hope that the information generated by this project will provide insights into methods to manage the development of the H7N9 outbreak and help to avert similar situations from arising.

A8 The epidemiology and evolution of influenza A/H1N1 and A/H3N2 virus from 2010 to 2015, in Ho Chi Minh City, Vietnam

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Influenza A viruses are highly infectious pathogens that constantly circulate in many animal hosts including humans, birds, pigs, horses and dogs. Infections with influenza viruses result in protective immunity mediated by antibodies against the viral surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). However, human and some avian influenza viruses have continuously undergone antigenic evolution to evade pre-existing host immunity, a phenomenon known as antigenic drift (accumulation of point mutations in HA and NA antigens). Antigenic drift explains the occurrence of repeated seasonal influenza epidemics in humans. In order to determine the virus' attack rate, cross-sectional seroprevalence studies are necessary. Typically, these seroprevalence studies of influenza viruses rely on HI (Hemagglutinin Inhibition assay) or MN (microneutralization test) to measure antibody titers in serum samples; these tests can have low sensitivity and they normally offer binary results (past infection, or not). Here, we conducted a seroprevalance study of influenza virus A/H1N1 and A/H3N2 in Ho Chi Minh City population between 2010 and 2015 using a novel protein microarray to measure the continuous-scale antibody titers against these subtypes in 5000 serum samples. Our platform is high-throughput, reproducible and can provide more informative antibody titers than previous assays. We also perform whole-genome sequencing of 190 influenza strains to investigate the antigenic changes of these subtypes over the study period. Tracking the antigenic changes of the viruses couple with a measurement of antibiotic response against these viruses in a population is an ideal data set to understand the evolution and immuno-epidemiology of these important pathogens and to provide timely and accurate data for the selection of vaccine strains.