

## Report

# Avian Influenza Viruses In China

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### Introduction

Natural infections with influenza A viruses have been reported in a variety of animal species including humans, pigs, horses, sea mammals and birds. Occasionally devastating pandemics occur in humans. In the 20th century the sudden emergence of antigenically different strains transmissible in humans, termed antigenic shift, has occurred on four occasions, 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1), each time resulting in a pandemic. Genetic analysis of the isolates demonstrated that 'new' strains most certainly emerged after reassortment of genes of viruses of avian and human origin in a permissive host.<sup>1</sup> The four human influenza pandemics occurred and resulted in significant mortality and morbidity; genetic evidences indicate that these pandemic strains were partially or entirely derived from avian influenza viruses (AIV).<sup>2,4</sup> Avian influenza virus involving at least three subtypes, H5, H7, and H9, has emerged as an important pathogen in the poultry industry and is of major current global health concern.<sup>5</sup> The first case report of chicken-to-human transmission was in Hong Kong in 1997;<sup>6</sup> since 2003, H5N1, a highly pathogenic AIV, has emerged in 10 Asian countries, including Thailand, Vietnam, and China, and has claimed at least 53 human lives.<sup>7</sup> Although viruses of relatively few HA and NA subtype combinations have been isolated from mammalian species, all 15 HA subtypes and all 9 NA subtypes, in most combinations, have been isolated from birds.<sup>1</sup>

It may be that infection of humans with avian influenza viruses occurs much more frequently than originally assumed, but due to their limited effect go unrecognized. For the human population as a whole the main danger of direct infection with avian influenza viruses appears to be if people infected with an 'avian' virus are infected simultaneously with a 'human' influenza virus. In such circumstances reassortment could occur with the potential

emergence of a virus fully capable of spread in the human population, but with antigenic characteristics for which the human population was immunologically naive. Presumably this represents a very rare coincidence, but which could result in a true influenza pandemic. The intra-pandemic influenza epidemics may have a substantial impact on a given population as a result of significant mortality, especially among the elderly and other vulnerable groups, and the severe economic cost associated with debilitating illness in a large portion of the population. Occasionally the degree of antigenic drift is sufficient that a very large proportion of the population is susceptible and severe epidemics occur with world-wide spread.<sup>1</sup>

### The H5N1 incident of China

Avian influenza viruses are zoonotic agents recognized as a continuing threat to both veterinary and human public health. During the past years, infection of humans with avian influenza viruses of three subtypes (H5, H7, and H9) has been detected on multiple occasions. In 1997, H5N1 avian influenza viruses transmitted from birds to humans in Hong Kong caused the deaths of 6 of 18 infected persons. The virus was eradicated by the slaughter of all poultry in Hong Kong, but new genotypes of H5N1 virus continued to emerge in poultry in Hong Kong in 2000 and 2001, and in 2003, antigenically and biologically novel H5N1 influenza virus killed one of two infected humans.<sup>8</sup>

An influenza virus (H5N1) known to infect only birds previously was found to infect human causing disease and death in Hong Kong in 1997 and the outbreak involved 18 patients with six deaths. Prior to the human outbreak, the H5N1 virus was found to cause extensive death in chickens in three farms in Hong Kong. Out of the 18 cases of human infection, eleven had severe infection with symptoms of pneumonia and multi-organ failure. All severe cases presented with lower respiratory infection and



lymphopenia and six eventually died. Case-fatality ratio was high among patients over 12 years of age (five out of nine). The occurrence of these cases was distributed over different part of Hong Kong with high population density. Of the 18 cases, 10 were females. Nine out of the 18 cases were children of 10 years age or younger. Twelve cases had a history of exposure to poultry. The index child had contact with chickens and ducklings at school. Three cases purchased poultry from the market before onset of illness and in another two cases, live chicken outlet was present nearby their home in which H5N1 virus was isolated during investigation of those two cases. Five other cases had regular visits to the markets and also live chicken outlets and one worked in the market.<sup>9</sup>

Genetic analysis revealed those human isolates of H5N1 subtype to be indistinguishable from a highly pathogenic avian influenza virus that was endemic in the local poultry population. This outbreak provided the first evidence that avian viruses could be transmitted directly to humans, without prior reassortment in a mammalian host or with a human virus, and could cause severe disease. Fortunately, the H5N1 virus transmitted to humans in 1997 did not develop the capacity for human-to-human transmission.<sup>10</sup>

In 2000–2002, H5N1 avian viruses reappeared in the poultry markets of Hong Kong, although they have not infected humans. In February 2003, an avian H5N1 virus closely related to one of those viruses was isolated from two humans with acute respiratory distress, one of whom died. Antigenic analysis of the new avian isolates showed a reactivity pattern different from that of H5N1 viruses isolated in 1997 and 2001. This finding suggests that significant antigenic variation has recently occurred among H5N1 viruses. Continued circulation of H5N1 and other avian viruses in Hong Kong raises the possibility of future human influenza outbreaks. Moreover, the acquisition of properties of human viruses by the avian viruses currently circulating in southeast China might result in a pandemic.<sup>11</sup>

Many factors may have contributed to the sudden outbreak of H5N1 virus in Hong Kong. These may include:

- the traditional Chinese requirement of purchasing live chicken for various cultural activities and the believe in the freshness of recently slaughtered birds;
- the crowded conditions of Hong Kong and the close proximity of live birds markets to residential complexes;
- the unhygienic way of slaughter and crowded conditions where live bird were kept in these markets;
- the close proximity of various poultry in traditional markets and wholesale markets where different species of birds were kept.

Under such conditions, the virus can be easily spread from one species of bird to another and eventually to human.<sup>9</sup>

Under the nationwide surveillance system established in July, 2004, patients admitted with pneumonia of unexplained origin are reported to the Chinese Center for Disease Control and Prevention. On Oct 8, 2005, a previously healthy 12-year-old girl in rural Hunan, China, developed fever, sore throat, and cough. She died of acute respiratory distress syndrome, disseminated intravascular coagulation, and multiorgan distress syndrome on Oct 17. In the meantime, her 9-year-old brother developed fever and cough on Oct 10. He was admitted to hospital on Oct 17 where he responded to treatment including amantadine, ribavirin, corticosteroids, and broad spectrum antibiotics; he was discharged on Nov 12. At his final follow-up on Dec 9, 2005, he remained asymptomatic. Only one serum specimen taken 8 days after onset of symptoms could be obtained from the girl, who was negative for H5-specific antibodies in both microneutralisation and haemagglutination-inhibition assays against the A/Hunan-Xiangtan-he/12/2005 virus, which was isolated from the only live chicken remaining in the household. The boy's samples, collected on days 8, 17, and 22 after the onset of the illness, showed a 4-fold or greater rise in antibody titre. Throat swabs for RT-PCR were, however, negative. The 12-year-old girl in the cluster is the first clinically diagnosed case of human H5N1 infection reported in mainland China, 8 years after the first documented outbreak in Hong Kong. Clinically, the respiratory distress in the infected children was similar to that reported elsewhere, and human-to-human transmission appeared to be inefficient. It is plausible that our cases had acquired the virus from diseased poultry rather than from one another.<sup>12</sup>

### The H9N2 incident of China

In March 1999, two independent isolations of influenza virus subtype H9N2 were made from girls aged one to four who recovered from flu-like illnesses in Hong Kong. Subsequently, five isolations of H9N2 virus from humans on mainland China in August 1998 were reported. H9N2 viruses were known to be widespread in poultry in China and other Asian countries. The H9N2 variant isolated from humans on the mainland also was isolated from pigs in southern China in 1999, but there was no serologic evidence that a stable virus lineage had become established in pigs or humans. The H9N2 viruses have continued to circulate in poultry throughout Europe and Asia, and are now considered to be enzootic throughout the entire region.<sup>8</sup>

The low pathogenic properties to poultry make these viruses easy to be widespread and transmitted to a new host; their gradually acquired ability to infect and replicate in mammalian and the fact that some genotype viruses are widespread and established in the pig population in China rank H9N2 viruses on the top of the list to cause human influenza pandemics in the future. The antigenic diversity and the resistance to the adamantanes of those viruses will pose great difficulties for future human influenza control



caused by H9N2 viruses. Therefore, urgent attention should be paid to the control of H9N2 influenza viruses in animals and to human's influenza pandemic preparedness.<sup>2</sup> The investigation indicating that close contact with the index case and exposure to poultry were at risk of being infected.

### The Evolutionary and Transmission Pathway for Avian Influenza Viruses

The influenza virus genome has remarkable plasticity because of a high mutation rate and its segmentation into 8 separate RNA molecules. This segmentation allows frequent genetic exchange by segment reassortment in hosts co-infected with two different influenza viruses.<sup>13</sup> This means that when two viruses infect the same cell, progeny viruses may inherit sets of RNA segments made up of combinations of segments identical to those either of the parent viruses. This gives a theoretical possible number of 28 (=256) different combinations that can form a complete set of RNA segments from a dual infection, although in practice only a few combinations possess the correct gene constellation required for viability. These 'new' genes almost certainly came from an influenza virus of avian origin and this led to the suggestion that antigenic shift occurred as a result of reassortment of genes in dual infections with viruses of human and avian origin. However, no natural infections of humans with avian viruses had been reported and it was clear that there was some barrier to the establishment of avian influenza viruses in the human population that was related to one or more of the gene segments.<sup>1</sup>

The transmission of H5N1 "bird flu" to humans in 1997 first established the ability of avian influenza viruses to be transmitted to humans despite their preferential binding to avian sialic acid receptors. There is limited serological evidence of H5N1 infection of humans and limited evidence of human-to-human transmission of H5N1 viruses. The available evidence suggests that since 1997, H5N1 viruses in China have caused limited severe or lethal human infection but have not been transmitted from human to human.<sup>8</sup>

### Conclusion

There is currently great concern about the spread of H5N1 viruses in China and possibly further. Their transmission to humans in Hong Kong in 1997 and in southern China in 2005 emphasizes the risk that this avian influenza subtype represents for human health and the importance of continued characterization of the virus subtypes in poultry and other fowl that live in close proximity to humans. Even if these new viruses do not appear to be transmitted efficiently from human to human, the appearance of new strains brings novel avian influenza viruses into contact with human influenza viruses, with the distinct possibility of reassortment and acquisition of the genes necessary for efficient human transmission. Such an event could result in the emergence of new pathogenic human influenza viruses. It is therefore crucial to gain a good understanding of the natural history and pathogenesis of avian influenza A viruses, particularly highly pathogenic viruses such as the H5N1 subtype.<sup>10</sup>

Given the abundance of influenza viruses in nature and the influenza H5N1 virus incidents in China it is increasingly clear that pandemic influenza is not only a zoonosis but also a non-eradicable zoonosis.<sup>14</sup> An effective H5N1 vaccine is a public health priority and the cornerstone for pandemic prevention and control. Reverse genetics approaches allow the rapid production of high-growth PR8 reassorted viruses by engineering a virus with a homologous HA gene lacking the polybasic amino acids associated with high virulence. Those candidate H5N1 pandemic vaccine viruses have been made available to vaccine manufacturers to produce pilot lots for clinical trials and are available for possible large-scale manufacturing should the need arise.<sup>13</sup>

The influenza virus continues to evolve, and new antigenic variants (drift strains) emerge constantly, giving rise to yearly epidemics. In addition, strains to which most humans have no immunity appear suddenly, and the resulting pandemics vary from serious to catastrophic.<sup>15</sup> Pandemic flu could be the biggest challenge that society will face. The world population is now much bigger and domestic and international travel is more extensive. Because all known influenza A subtypes exist in the aquatic bird reservoir, influenza is not an eradicable disease; prevention and control are the only realistic goals. The optimal strategy for control of pandemic influenza is early intervention with a vaccine, produced, ideally, from the actual pandemic strain or at least from an antigenically well-matched strain.<sup>16</sup> Therefore, it is necessary to evaluate how well the prevailing isolates match the present vaccine and update the vaccine formulation accordingly.<sup>17</sup>

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