

Ecology and Evolution of Influenza A (H5N1) Virus in Asia: Evidence From Systematic Influenza Surveillance

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The development of highly pathogenic avian influenza A (H5N1) viruses in poultry in Eurasia accompanied with the increase in human infection in 2006 and the recent reemergence of poultry outbreaks throughout the region, suggests that the virus has not been effectively contained and that the pandemic threat persists. Virological and epidemiological findings from our surveillance in live-poultry markets conducted over the last seven years in southern China has provided a comprehensive view of the ecology and evolution of H5N1 influenza viruses and revealed that they are endemic in different types of market poultry. Genetic and antigenic analyses have also demonstrated the dynamic evolution of these viruses in the region, with repeated introductions from southern China to neighboring regions, including Cambodia, Indonesia, Malaysia, Thailand, and Vietnam. A further dramatic spread of the virus westwards throughout Central and Southern Asia, Europe and Africa was also seen after detection of an initial outbreak in wild birds at Qinghai Lake in Central China, in line with our predictions. Genetic analyses revealed that after the H5N1 influenza viruses had established and been endemic several years, they developed into regionally distinct sublineages that allowed us to further trace their transmission pathways. However, recent surveillance data has revealed the emergence and predominance of a single H5N1 virus sublineage in poultry since late 2005 that has gradually replaced those previously detected multiple regional distinct sublineages in China. These viruses have already transmitted to neighboring regions thereby resulting in a new transmission and outbreak wave in Southeast Asia. The persistence of H5N1 virus since it first caused human disease in Hong Kong 11 years ago, as demonstrated by its endemicity over a large geographical region, along with repeated disease outbreaks in both poultry and humans indicates that it will be a long-term and difficult task to bring this virus under control in the absence of well developed disease control systems.

Introduction

Extensive surveillance and genetic studies have revealed that highly pathogenic avian influenza H5N1 viruses have become first predominant and then endemic in poultry in southern China and Southeast Asia since 2003 [1]. This resulted in the establishment of multiple distinct regional sublineages [2]. The recognition of multiple different H5N1 sublineages

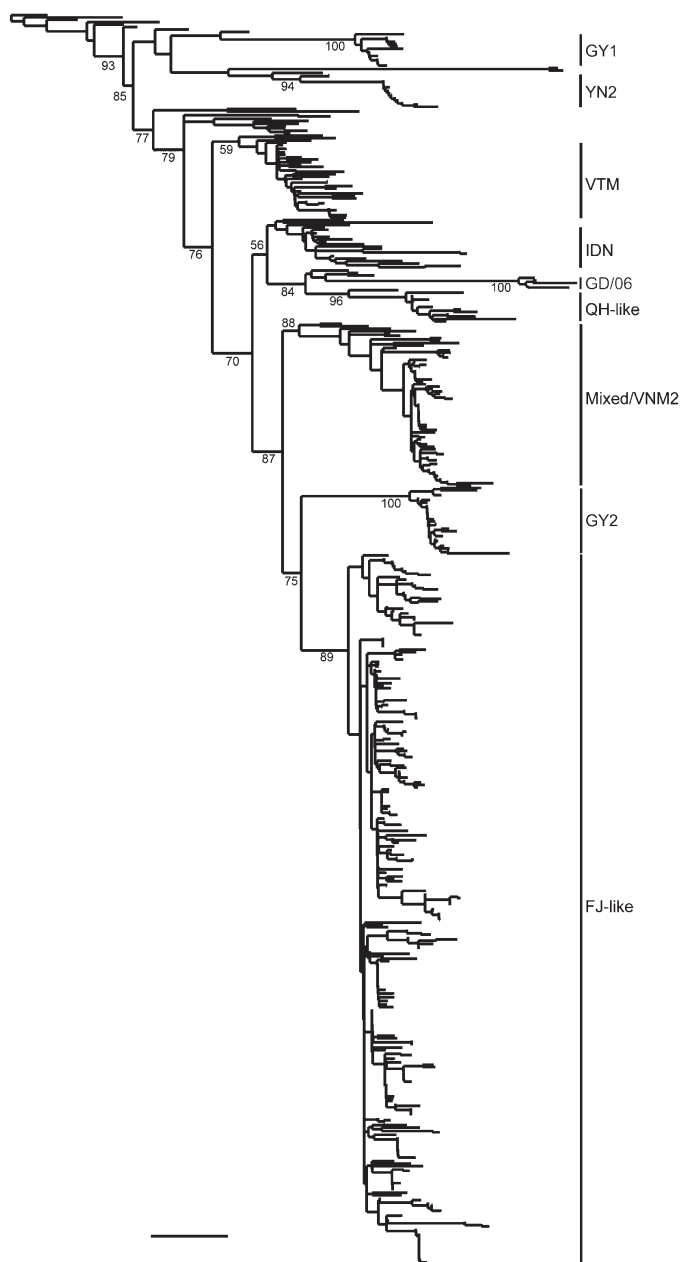
makes it possible to identify the source, and to understand the evolutionary and transmission pathways of those H5N1 viruses that have become widespread in Southeast Asia, Europe and Africa. Since H5N1 influenza virus caused the first outbreak in migratory waterfowls at Qinghai Lake in May 2005 [3], a new transmission and outbreak wave was initiated. The virus expanded its geographical distribution and caused outbreaks in poultry in over 60 countries from Central Asia, the Middle East, Europe and Africa [4]. This led directly to a marked increase in human infection cases and escalated the pandemic threat. From Jan 2006 to June 2007 the World Health Organization (WHO) confirmed 170 cases from 12 countries; while for the period 2003 to 2005 there was a total of 148 cases from just five countries [5]. Also, in Indonesia there were suspected cases of human-to-human transmission involving members of an extended family and the infection sources of other human cases have not been identified [6]. In China, despite a compulsory program for the vaccination of all poultry commencing in September 2005 [7], H5N1 influenza virus has caused outbreaks in poultry in 12 provinces from October 2005 to August 2006 [4]. At the same time, twenty-two human infection cases have been confirmed from 14 provinces since November 2005 [4, 5]. Some of those cases were residents of metropolitan areas remote from poultry farms, such as Guangzhou, Wuhan and Shanghai [4]. Furthermore, there were no obvious poultry outbreaks reported in neighboring markets or farms before or after the onset of those human infections. So whether those people were infected locally and directly from affected poultry or other sources, including humans, is still unknown. This situation directly challenges current pandemic preparedness plans, raising concern that a pandemic could emerge not only from countryside but also from an urban area, just as SARS emerged from the live-animal markets of Guangzhou and the Pearl River Delta [8, 9].

Results

Surveillance. From July 2005 to June 2006 our ongoing influenza surveillance in live-poultry markets in six provinces of southern China showed that 1,294 of 53,220 (overall isolation rate 2.4%) poultry were H5N1 positive. The main body of H5N1 isolates was from duck and goose, with only a small number isolated from chicken (chicken 0.5%, duck 3.3%, goose 3.5%). The prevalence of H5N1 viruses in southern China has increased when compared to the period July 2004 to June 2005 (overall 0.9%, chicken 0.2%, duck 1.3%, goose 2.0%). A winter-seasonal peak was observed from October 2005 to March 2006 as in previous years [1, 2]), during which H5N1 influenza viruses were isolated in each province tested. However, an extension of the peak season was observed in April to June 2006 as isolation rates remained high in these warmer months. Comparison between different types of poultry shows that H5N1 viruses were mainly isolated from domestic duck and goose wherein the viruses were prevalent year-round, while chicken tested positive mostly during the winter. It is notable that in the last 12 months, H5N1 positive chicken were detected in 11 months,

a marked increase from only four positive months in 2004/05 (data not shown). These findings indicate an escalation of H5N1 activity in poultry in 2005/06 compared to previous years. These findings suggest that H5N1 influenza viruses have not been effectively contained in this region and have maintained endemicity broadly in poultry, especially domestic duck and goose.

Figure 1. Phylogenetic relationships of the HA genes of representative influenza A viruses isolated in Asia. Trees were generated by the neighbor-joining method in the PAUP* program. Numbers above or below branches indicate neighbor-joining bootstrap values. Not all supports are shown due to space constraints. Analysis was based on nucleotides 1–1011 and the tree was rooted to A/Tern/South Africa/61. Scale bar, 0.01 substitutions per site.



Phylogenetic Analysis. To better understand the increased prevalence of H5N1 in poultry and the emergence of human infection in China, 390 (30% of total new isolates) of those avian H5N1 influenza viruses isolated from July 2005 to June 2006, plus 16 viruses isolated from smuggled poultry and dead wild birds in Hong Kong in early 2006, were sequenced and analyzed together with sequences available from public databases. Phylogenetic analysis of the hemagglutinin (HA) gene revealed that 266 of 390 (68%) of those recent H5N1 viruses from southern China, formed a distinct H5N1 sublineage (Fujian (FJ)-like, Clade 2.3.4) (Figure 1). Twenty-eight viruses isolated in Guiyang from November 2005 to January 2006 formed a sublineage (GY2, Clade 2.3.3) that is the sister-group to the Fujian-like sublineage (Figure 1). Another three sublineages from Guangdong (GD/06, Clade 2.5, n=6), Guiyang (GY1, Clade 4, n=14) and Yunnan (YN2, Clade 7, n=13) were also identified. A further 59 viruses grouped in the Mixed/VNM2 sublineage (Clade 2.3.2), while only a single virus (Gf/ST/1341/06) belonged to the QH-like sublineage (Clade 2.2) currently circulating in Africa and Europe (Figure 1). The remaining viruses analyzed in this study belonged to previously reported sublineages from China and Southeast Asia, except two isolates from Hunan (Ck/HN/2246/06 and Ck/HN/2292/06), isolated in May 2006 that do not fall with any of these sublineages. The prototype virus of the FJ-like sublineage (Dk/FJ/1734/05) was detected in March 2005. From July to September 2005, only a single strain of 33 sequenced viruses was FJ-like (Table 1).

Table 1. Number of viruses from the Fujian-like sublineage in China.

Collection date		Number of Fujian-like viruses*
2005	Jul-Sep	1/33 (3)
	Oct-Dec	72/136 (53)
2006	Jan-Mar	90/113 (80)
	Apr-Jun	103/108 (95)
Total		266/390 (68)

Remarkably, from October 2005 onwards the percentage of FJ-like viruses detected increased dramatically, until from April to June 2006, 103 of 108 (95%) of H5N1 poultry isolates tested were FJ-like (Table 1). Viruses from other sublineages were not detected in our surveillance since October 2005, November 2005, March 2006, April 2006 and May 2006, respectively. These findings reflect the process of FJ-like viruses gradually becoming predominant in this region. Phylogenetic analysis also revealed that the HA gene of five recent human H5N1 viruses from different provinces of China [4, 10, 11] belong to this FJ-like sublineage and were most closely related to poultry isolates. This suggests that H5N1 human infection from China since November 2005 were infected directly from affected poultry. Furthermore, H5N1 viruses isolated in early 2006 from neighboring regions of mainland China, including the 16 Hong Kong viruses and two poultry isolates from Laos and Malaysia, all joined the FJ-like sublineage.

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Discussion

The emergence of this FJ-like sublineage has had similar consequences to the first wave of virus transmission throughout Southeast Asia in early 2004 [1] and the second wave to Europe and Africa that followed the Qinghai Lake H5N1 outbreak [3, 4]. The findings of our study show that this virus has replaced most of those previously established regional sublineages across a large geographical area in China [2]. The predominance of this FJ-like virus appears to be responsible for the increased prevalence of H5N1 in poultry since October 2005 and recent human infection cases in China [4, 5]. Furthermore, it has already caused poultry outbreaks in Laos, Malaysia, Thailand and Vietnam [4, 5]. As such, this variant has already initiated a third wave of transmission throughout Southeast Asia, and may spread further in Eurasia. It is also probable that this virus will continue to evolve to form other regionally distinct sublineages as witnessed with the H5N1 genotype viruses in the first and second transmission waves [1, 2]. Previously we have described the establishment of multiple sublineages of H5N1 virus in southern China and Southeast Asia [2]. The emergence and replacement of these sublineages by FJ-like viruses within a short period of time highlights the difficulties faced in controlling H5N1 virus in China. A complex ecology and highly diverse virus populations make it almost impossible to capture each circulating virus sublineage even with the application of mass vaccination. This has resulted in recurrent H5N1 outbreaks in poultry in different regions and led to occasional human infection.

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