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The emergence of influenza A H7N9 in human beings 16 years after influenza A H5N1: a tale of two cities

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Infection with either influenza A H5N1 virus in 1997 or avian influenza A H7N9 virus in 2013 caused severe pneumonia that did not respond to typical or atypical antimicrobial treatment, and resulted in high mortality. Both viruses are reassortants with internal genes derived from avian influenza A H9N2 viruses that circulate in Asian poultry. Both viruses have genetic markers of mammalian adaptation in their haemagglutinin and polymerase PB2 subunits, which enhanced binding to human-type receptors and improved replication in mammals, respectively. Hong Kong (affected by H5N1 in 1997) and Shanghai (affected by H7N9 in 2013) are two rapidly flourishing cosmopolitan megacities that were increasing in human population and poultry consumption before the outbreaks. Both cities are located along the avian migratory route at the Pearl River delta and Yangtze River delta. Whether the widespread use of the H5N1 vaccine in east Asia—with suboptimum biosecurity measures in live poultry markets and farms—predisposed to the emergence of H7N9 or other virus subtypes needs further investigation. Why H7N9 seems to be more readily transmitted from poultry to people than H5N1 is still unclear.

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See **Online** for appendix

1997 and 2013 avian influenza viruses

The H5N1 virus caused the first human outbreak of avian influenza in Hong Kong in 1997.[1] Over the next 16 years, up to June 2013, more than 600 human cases with 60% mortality have been reported from mainland China, southeast Asia, the Middle East, Africa, and Europe.[2,3] Other avian influenza viruses belonging to subtypes H6, H7, H9, and H10 have also crossed between species and caused mostly sporadic non-fatal cases in human beings; one fatal case of influenza A H7N7 was reported in the Netherlands.[4–9] In March 2013, the first case series of human infections with a novel avian H7N9 virus was reported in China (table 1).[10] By June 26, 2013, 132 symptomatic cases and one asymptomatic case with 40 deaths had been reported (appendix). Severe infections in human beings were characterised by rapidly progressive pneumonia acquired in the community, multiorgan dysfunction, and cytokine dysregulation.[11–15] The initial cases of infections in human beings were clustered around the lower Yangtze River delta, including Shanghai, Jiangsu, Zhejiang, and Anhui. Subsequently, cases were identified in other areas of China, including Beijing, Henan, Shandong, Jiangxi, Hunan, and Fujian, which are located along an avian migratory pathway (figure 1).[18] On April 24, 2013, the first case outside the mainland was confirmed in a 53-year-old Taiwanese businessman who regularly travelled between Jiangsu and Taiwan.[13] We compare the geographical and social characteristics between Hong Kong and Shanghai, the likely origin and virological features of the 1997 influenza A H5N1 virus and the 2013 influenza A H7N9 virus, and clinical manifestations caused by these two viruses.

Generation of novel avian influenza viral reassortants

The influenza viruses belong to the RNA virus family Orthomyxoviridae. On the basis of antigenic and genetic differences between the internal proteins, nucleoprotein and matrix protein, influenza viruses are classified into A, B, and C. The influenza A virus has a negative-sense, single-stranded, and eight-segmented genome. On the basis of two surface proteins, 17 haemagglutinin subtypes and ten neuraminidase subtypes can be found (figure 2).[19] Except H17 and N10, which have been found in bats only, all haemagglutinin and neuraminidase subtypes have been found in wild waterfowl.[20] Unlike other neuraminidase subtypes, N10 does not have neuraminidase activity.[21] The 17 haemagglutinin subtypes are further classified into group 1 and group 2 on the basis of the antigenic and phylogenetic characteristics of haemagglutinin. The HA1 subunit of the haemagglutinin attaches onto host cell sialic acid receptor. The haemagglutinin precursor protein HA0 is cleaved by proteases into HA1 and HA2 before HA2 can mediate virion–cell fusion. The internal proteins are encoded by six other gene segments encoding nucleoprotein NP, matrix protein M1, ion channel protein M2, the polymerase complex proteins PB1, PB2, PA, including PB1-F2, PA-X, PA-N155, PA-N182, and non-structural proteins including PB1-F2, PA-X, PA-N155, PA-N182, NS1, and NS2.[19,22]

The eight gene segments can reassort when two different viruses infect an animal host cell. Phylogenetic analysis shows that the 1997 H5N1 virus infecting human beings was a reassortant with goose H5N1 haemagglutinin, teal H6N1 neuraminidase, and internal genes from quail H9N2 and teal H6N1.[23,24] The 2003 H7N7 virus that infected human beings in the Netherlands was a reassortant between avian influenza A H7N3, influenza A H10N7, and other Eurasian avian influenza viruses (figure 3).[9] On the basis of available sequences in the public domain, the 2013 H7N9 virus is also a reassortant of avian influenza viruses consisting of haemagglutinin and neuraminidase most closely related to H7N3 viruses isolated from ducks in Zhejiang and H7N9 viruses from wild birds in Korea, respectively. The M, NP, and NS genes are most closely related to H9N2 viruses from chickens in eastern China, whereas

	Place or organisation	Event
Feb 18, 2013	Shanghai	Case one: symptom onset
March 4, 2013	Shanghai	Case one: died
March 10, 2013	Shanghai	Case two: died
March 13, 2013	Shanghai	Cases 73–77: symptom onset (retrospectively diagnosed on April 16, 2013); cases one and 76 represent first family cluster; another son of case one also died without virological diagnosis
March 31, 2013	Shanghai Anhui Chinese Centre for Disease Control and Prevention, China	Cases one and two (both fatal) reported First case in Anhui (case three) reported The China Health and Family Planning Commission notified the WHO of three cases of human infection with influenza A H7N9 (cases one to three)
Aprilil 2, 2013	Jiangsu	First four cases in Jiangsu (cases four to seven) reported
April 3, 2013	Zhejiang	First two cases in Zhejiang (cases eight and nine) reported
April 4, 2013	Shanghai	First mild case (case 14) reported; one market pigeon positive for H7N9 virus
April 5, 2013	Shanghai	Culling of 20 536 poulty: 19 poultry and environmental samples from three markets positive for H7N9 very similar to pigeon isolate
April 6, 2013	Ministry of Health, China Shanghai, Jiangsu, and Zhejiang	Approval of peramivir as anti-influenza drug in China Suspension of live poultry trading; culling of >20 000 live birds
April 8, 2013	Ministry of Health, China	Development of vaccines for H7N9 announced
April 10, 2013	Shanghai Jiangsu, Zhejiang, Anhui	Case 14 recovered and discharged from hospital Duck and chicken samples positive for H7N9
April 13, 2013	Beijing Shanghai	First case outside the Yangtze River delta (case 44) reported Second cluster in family: case 47 and case 13
April 14, 2013	Henan	More cases outside the Yangtze River delta (cases 50 and 51) reported
April 15, 2013	Beijing	Third cluster: the first asymptomatic carrier (case A1) detected during contact tracing of case 44
April 18, 2013	Henan	Another case outside the Yangtze River delta (case 83) reported
April 19, 2013	World Health Organization	Fourth cluster in family announced
April 23, 2013	Shandong	First case in Shandong (case 105) reported
April 24, 2013	Taiwan	First case outside the mainland (case 109) reported
April 25, 2013	Jiangxi	First case in Jiangxi (case 113) reported
April 26, 2013	Fujian	First case in Fujian (case 115) reported
April 27, 2013	Hunan	First case in Hunan (case 120) reported
May 5, 2013	China	A total of 51 poultry and environmental samples from multiple regions were positive for H7N9
As of June 26, 2013	World Health Organization	A total of 132 symptomatic cases with 40 fatalities reported globally

See appendix for details of individual cases.

***Table 1*: Important events in the influenza A H7N9 outbreak in 2013**

the PA, PB1, and PB2 genes are most closely related to those of H9N2 viruses from brambling in Beijing.[11,12,13,25,26] Because the origin of all gene segments of the 2013 H7N9 virus are avian, gene reassortment probably took place in an avian host. Although reassortment events of avian influenza viruses have been recorded in pigs in Korea,[27] the 2013 H7N9 virus and the immediate precursors have not been found in pigs.[28]

The haemagglutinin sequence of one of the 2013 H7N9 virus human isolates (A/Shanghai/1/2013) was phylogenetically distinct from other human and avian isolates,[12,29] suggesting that this new reassortant might have been circulating for some time to achieve this degree of diversity. Because of the roughly 5% nucleotide differences in the H7 and N9 genes between the 2013 H7N9 virus and the closest avian virus genes, more extensive virological surveillance of wild or domestic avian and non-avian animal species, as has been done for the Severe acute respiratory syndrome coronavirus,[30] is needed to understand the evolutionary pathway of this H7N9 virus, which is the first N9 subtype virus infecting human beings. Up to now, only one wild pigeon from Nanjing has tested positive for the 2013 H7N9 virus,[31] but the epidemiological and genomic details of this virus isolate are not yet available.

Not all human disease preceded by poultry outbreaks

One unique feature of the 2013 H7N9 human epidemic is the absence of preceding die-offs in poultry or wild birds, which makes epidemiological control difficult. The start of the H5N1 outbreak in 1997 was marked by three highly pathogenic avian influenza virus outbreaks on farms 1 month before the first human case and about

8 months before the next 17 human cases.[32] Avian influenza virus can be divided into highly pathogenic avian influenza virus and low pathogenic avian influenza virus on the basis of pathogenicity in chickens. The World Organization for Animal Health has defined highly pathogenic avian influenza virus as any influenza virus that is lethal for six or more of eight 4–8-week-old susceptible chickens by 10 days after intravenous inoculation with 0·2 mL of a 1/10 dilution of a bacteria-free infective allantoic fluid, or that has an intravenous pathogenicity index of greater than 1·2, which is calculated by scores on the severity of illness.[33] One key feature of highly pathogenic avian influenza virus is the presence of multibasic aminoacids at the haemagglutinin cleavage site, which render haemagglutinin susceptible to cleavage by many different proteases.[34] Hence, H5 or H7 viruses that have low pathogenicity in chickens are still regarded as highly pathogenic avian influenza virus if their HA0 cleavage sequence is similar to that of other highly pathogenic avian influenza viruses. The haemagglutinin cleavage site from all reported 2013 H7N9 viruses do not possess multibasic aminoacids.[11,12,35] Data from preliminary pathogenicity testing suggest that chickens and quails infected with H7N9 do not show signs of illness.[36] A low pathogenic avian influenza virus can mutate to become highly pathogenic during an outbreak in domestic poultry. For the H7N3 virus outbreaks in 2002 and 2004, the difference in the cleavage site between low pathogenic avian influenza virus and highly pathogenic avian influenza virus was speculated to be a sequence insertion from the neuraminidase or M gene at the haemagglutinin cleavage site through intersegmental recombination.[37] Close monitoring of the evolution of the 2013 H7N9 is needed.

Another possible explanation for the apparent absence of a preceding infection with H7N9 in poultry in the 2013 outbreak is that previous infection by a closely related low pathogenic avian influenza H7 virus elicited cross-protection for this H7N9 virus because H7N3 virus was recently reported in ducks in Zhejiang, China.[38] Furthermore, prevalent H9N2 infections in poultry or the extensive use of the H5N1 vaccine in poultry in China might induce cell-mediated immunity against highly conserved internal viral proteins without inducing any neutralising antibody. This process might be sufficient to militate against poultry death, but not asymptomatic viral shedding.[39] The effect of previous infection by other avian influenza virus subtypes and previous H5N1 vaccine on the susceptibility to infection by H7N9 deserves further investigation.

Virus adaptation for interspecies jumping

Interspecies jumping of an avian influenza virus into human beings is favoured by several factors: viral mutations that allow the virus to bind, replicate, and spread in human beings; a high inoculum of virus during the transmission between the poultry and human being; host susceptibility such as the presence of underlying diseases; extremes of age; and genetic predispositions. One key viral factor governing host adaptation and transmission is the relative binding affinity between haemagglutinin and the sialic acid receptor on the host cell surface.[40] Avian influenza viruses generally prefer α-2,3 sialic acid receptors abundantly found in avian alimentary tract and human influenza viruses generally prefer α-2,6 sialic acid receptors abundantly found in the human respiratory tract. H5N1 viruses can invade the lower respiratory tract of human beings because the viruses retain strong affinity for the α-2,3-linked receptors present in the lower respiratory tract of human beings.[41] An increased affinity for the α-2,6-linked receptors, which are abundant in the upper respiratory tract of human beings, is believed to be responsible for transmission between birds and human beings. In Egypt, which has had the highest number of H5N1 infections since 2009, 99% of virus isolates had a Thr160Ala (H3 numbering) mutation, which is associated with loss of glycosylation at position 158–160, and increased transmissibility in ferrets and guinea pigs.[42,43] Thr160Ala was also present in all reported strains of 2013 H7N9 (table 2). The haemagglutinin mutations Gly186Val and Gln226Leu, which increase the binding of H5N1 and H7 viruses to α-2,6-linked receptor and H5N1 virus transmission between ferrets, are present in most strains of the human and avian 2013 H7N9, but none of the 1997 H5N1 strains (table 2).[11,12,35,45–47] However, a recent structural modelling and binding study with human tracheal epithelium and

Figure 1: **Geographical areas with laboratory-confirmed cases of human infection by the avian influenza A H7N9 virus, as of June 26, 2013**

*One case in Beijing (case A1) was asymptomatic. †The details of six additional deaths were not available and thus not shown in the map. Data from references 16 and 17.

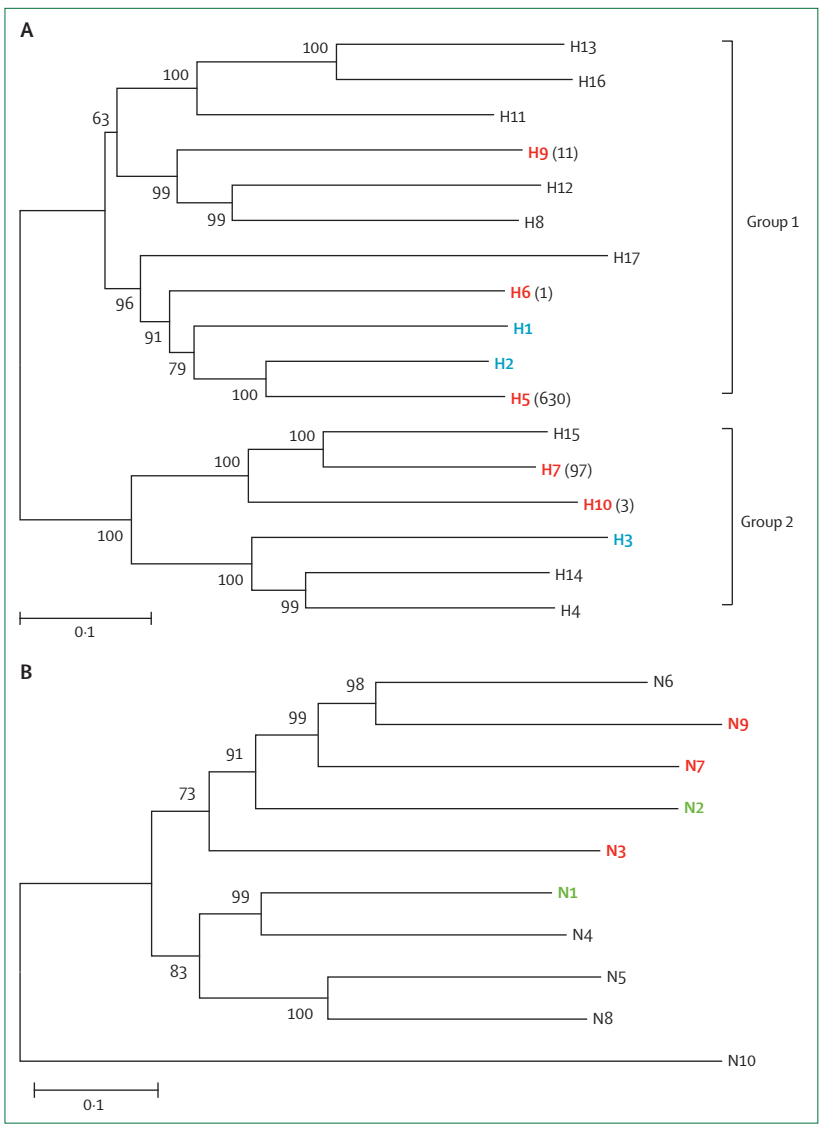

Figure 2: **Subtyping of influenza A virus by surface protein genes of influenza A virus**
(A) Phylogenetic tree of the haemagglutinin gene. (B) Phylogenetic tree of the neuraminidase gene. Subtypes in bold colour represent those that have been found in human beings. Subtypes in blue have caused human seasonal or pandemic influenza. Subtypes in red are avian-origin influenza. Subtypes in green have caused both human seasonal and pandemic influenza or avian-origin influenza. Number in parenthesis shows number of human real-time-PCR or culture-confirmed avian influenza infections reported, but not including the 2013 influenza A H7N9 cases. The sequences were retrieved from the NCBI database and the phylogenetic trees were constructed by the neighbour-joining method with bootstrap replication (1000 bootstraps) with MEGA 5.1. Numbers at nodes show levels of bootstrap support calculated from 1000 trees. Scale bars show the estimated number of substitutions per ten bases.

alveolar tissue sections suggested that 2013 H7N9 has weak binding to both α-2,3-linked and α-2,6-linked receptors.[48] Further studies should confirm whether various strains of H7N9 differ in binding affinities.

Neuraminidase enables release of virus particles from the host cell surface. Deletion in the stalk region of neuraminidase, which was found in the human H5N1 viruses and previous H7 viruses, is associated with increased virulence and replication in the respiratory tracts of chickens.[49] 2013 H7N9 also has a five aminoacid deletion in the stalk region, which might improve its adaptation to poultry.[11,12,29,35] Comparative studies in wild waterfowl and domestic chickens will ascertain the degree of adaptation by this virus in these avian species.

Mutations in the polymerase complex affect viral replication. PB2 Glu627Lys mutation is associated with increased viral replication at 33°C, the nasal body temperature in human beings, which is markedly lower than that of avian species.[50] Furthermore, this mutation is important in aerosol transmission of avian H1N1 virus between ferrets.[51] Glu627Lys was found only in human strains, but not the avian strains, of 1997 H5N1 or 2013 H7N9 (table 2). However, Glu627Lys was specifically found in the Qinghai outbreak of H5N1 in 2005.[52] PB2 Asp701Asn is another important mutation associated with mammalian adaptation and transmission.[42,53] Notably, most virus isolates from individuals infected with 2013 H7N9 had either Glu627Lys or Asp701Asn, but not both. Asp701Asn can compensate for the lack of Glu627Lys for transmission between guinea pigs.[53]

Other mutations affect viral replication, transmission, and virulence of influenza viruses. NS1 protein is the key virulence factor counteracting innate host immunity. NS1 protein contains a four-aminoacid motif at the C terminal that disrupts cellular signalling by binding to the host PDZ domain-containing proteins. Recombinant influenza virus with PDZ-binding motif has increased pathogenicity in mice.[54] NS1 protein from human seasonal influenza viruses cannot bind to human proteins containing PDZ domain.[55] The NS1 of all 2013 H7N9 strains has a deletion of the PDZ motif. The structural M1 protein is needed for viral assembly and budding. Two mutations in M1, Asn30Asp and Thr215Ala, associated with increased virulence in a mice model, are found in all strains of the 2013 H7N9 virus.[56]

Epidemiological characteristics

Before the 2013 outbreak, the H7N9 virus had never been reported to cause human infection, although other H7 viruses had been associated with sporadic infections since 1979 (table 3).[5,7,9,57–63] Serological evidence of H7 infection was present in 38% of a rural population in the Jiangsu Province near Shanghai.[64] However, a study including 1544 serum samples from poultry workers in eastern China in 2012 did not identify any seropositive samples by microneutralisation assay, suggesting that subclinical H7N9 infection was unlikely before 2013.[65]

Influenza A subtypes H7N1, H7N2, H7N3, H7N7, and H7N8 were previously isolated from poultry and wild birds from China.[38,66] Although studies of seroprevalence did not find any evidence of H7 infection among sparrows or pigs in China,[67,68] H7N9 viruses were isolated from birds in nearby countries, including Mongolia and South Korea in 2008.[69,70] Moreover, in the USA, H7N9 viruses have caused outbreaks in chickens.[71] Other H7N9 viruses were reported in wild birds or poultry from four continents.[10,55,72,73]

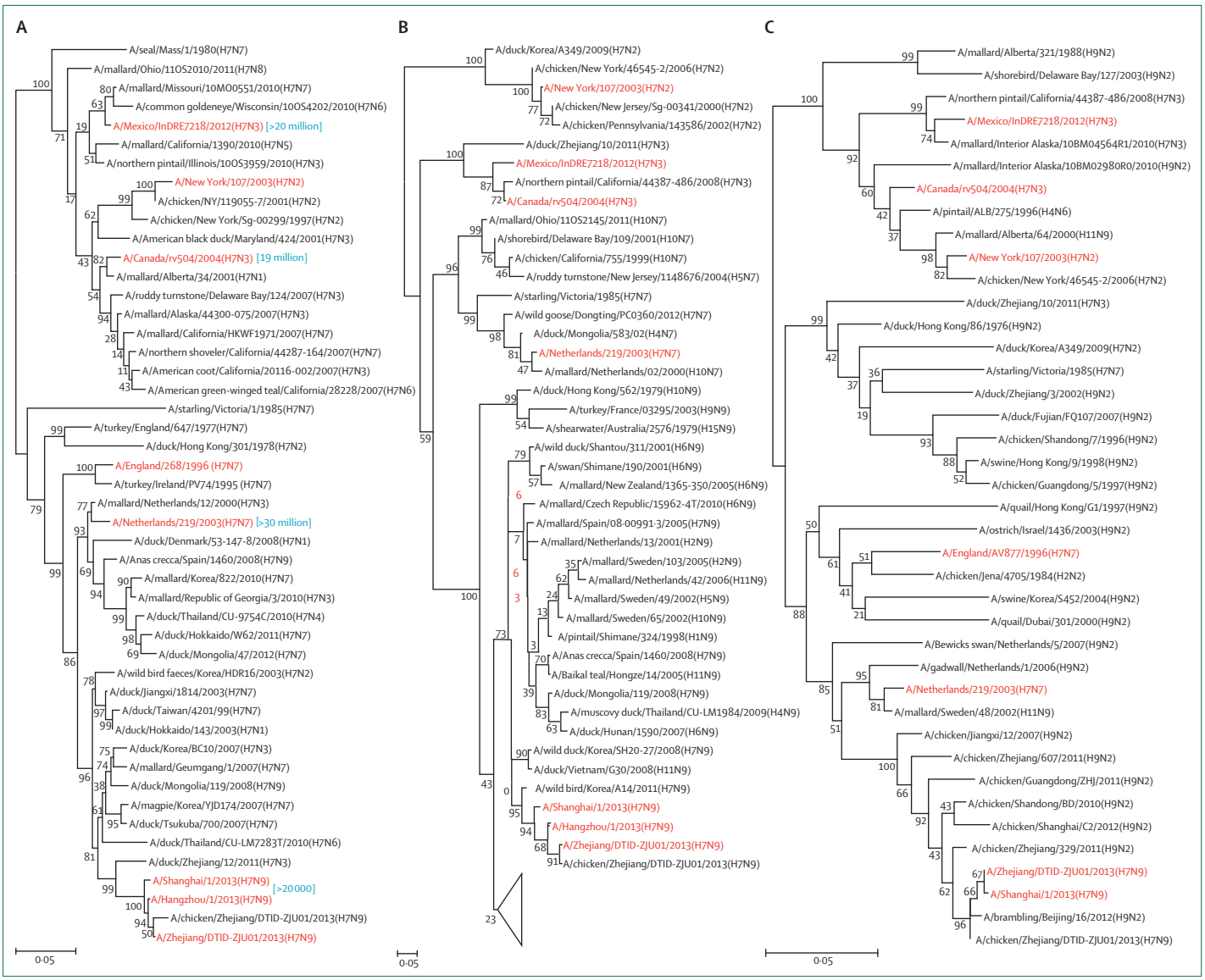

Figure 3. **Phylogenetic associations of avian H7 viruses that have been reported to cause human infections**
(A) Haemagglutinin gene. (B) Neuraminidase gene. (C) PB2 gene. The haemagglutinin, neuraminidase, and PB2 genes of the human isolates and the most closely related avian isolates, which are possible candidates as the gene source for reassortment, are shown. Only representative H7 isolates from human and the closely related isolates from avian species of preceding years are shown. The phylogenetic trees were constructed by the neighbour-joining method with bootstrap replication (1000 bootstraps) with MEGA 5.1. The neuraminidase gene sequence of 1996 influenza A H7N7 is not available in the public domain. Human strains are highlighted in red. Number of poultry culled is highlighted in blue. Scale bars show the estimated number of substitutions per 20 bases.

Most of the laboratory-confirmed cases of 2013 H7N9 were older adults living in the city, with a median age of 61 years. By contrast, in the 1997 H5N1 outbreak in Hong Kong, 61% of patients were younger than 18 years of age[1,74] and the mean age of patients with H5N1 in China between 2003 and 2008 was 28·1 years (table 4).[78] The severe disease in older adults and the small number of cases in children in the 2013 H7N9 outbreak can be accounted for by several factors. First, H7N9 infection in children was mainly mild or asymptomatic (appendix);[14,79,80] therefore, infection in this age group might have been underdiagnosed because confirmatory laboratory tests were usually reserved for severe cases. Two children were found to be infected with H7N9 during an enhanced surveillance that included 6333 children younger than 4 years of age with influenza-like illness.[79] Second, some adult patients were poultry workers; generally only adults go to live poultry markets and therefore adults might have had a greater dose or prolonged duration of exposure to H7N9 than did children. Additionally, adults might have an increased chance of exposure to pet birds. Finally, older adults might have impaired development of specific immune response to the H7N9 virus because of the immunological phenomenon of original antigenic sin.[81,82]

	Importance of the mutation	2013 influenza A H7N9		1997 influenza A H5N1	
		Human	Avian and environmental	Human	Avian and environmental
Haemagglutinin (H3 numbering)					
Ala135Thr	Increased viral replication	ND	ND	ND	ND
Asn158Asp	Mammalian transmission	ND	ND	ND	ND
Asn224Lys	Mammalian transmission	ND	ND	ND	ND
Gly228Ser	Mammalian transmission	ND	ND	ND	ND
Thr318Ile	Mammalian transmission	ND	ND	ND	ND
Ser138Ala	Ser variant associated with adaptation to pigs	9/10	All	All	All
Thr160Ala	Increased binding to α-2,6-linked sialic acid receptor	All	All	3 of 5	6 of 10
Gly186Val	Increased binding to α-2,6-linked sialic acid receptor	9 of 10	All	ND	ND
Gln226Leu	Increased binding to α-2,6-linked sialic acid receptor	8 of 10*	5 of 6	ND	ND
Multibasic aminoacid at HA0 cleavage site	Cleavage by ubiquitous proteases	ND	ND	All	All
Neuraminidase (viral release from host cell surface)					
Deletions in stalk region	Increased virulence	All	All	All	All
Arg292Lys	Neuraminidase resistance	2 of 11	ND	ND	ND
PB2 (viral replication)					
Leu89Val	Enhanced polymerase activity	All	All	All	All
Glu627Lys	Improved viral replication at 33°C	7 of 9	ND	2 of 9	ND
Asp701Asn	Mammalian adaptation	1 of 9	ND	ND	ND
PB1 (viral replication)					
His99Tyr	Enables droplet transmission in ferrets	ND	ND	ND	ND
Ile368Val	Enables droplet transmission in ferrets	5 of 7	All	ND	ND
PB1-F2 (induce cellular apoptosis and inhibit function of type I interferon)					
Full-length	Full-length PB1-F2 needed for virulence in mice	All	3 of 4	All	All
Asn66Ser	Increased virulence in a mice model	ND	ND	ND	ND
Matrix protein M1 (viral assembly and budding)					
Asn30Asp, Thr215Ala	Increased virulence in a mice model	All	All	All	All
Matrix protein M2					
Ser31Asn	Amantadine resistance	All	All	ND	ND
NS1 (counteracts host antiviral response)					
Pro42Ser	Increased virulence in mice	All	All	All	All
PDZ-binding motif	Signalling of host proteins	Deleted	Deleted	Avian type	Avian type

Aminoacid sequences of A/Zhejiang/DTID-ZJU01/2013(H7N9), A/Hangzhou/1/2013(H7N9), A/Hangzhou/2/2013(H7N9), A/Hangzhou/3/2013(H7N9), A/Shanghai/4664T/2013(H7N9), A/Nanjing/1/2013(H7N9), A/Fujian/1/2013, A/Taiwan/S02076/2013(H7N9), A/chicken/Zhejiang/DTID-ZJU01/2013(H7N9), A/environment/Hangzhou/34/2013(H7N9), A/environment/Nanjing/2913/2013(H7N9), and all 1997 influenza A H5N1 strains were obtained from NCBI Influenza Virus Resource or from references 11, 12,29,35, and 44. Aminoacid sequences of 2013 influenza A H7N9 sequences of human isolates from Shanghai or Anhui were obtained from Global Initiative on Sharing Avian Influenza Data (GISAID) database and references 12 or 35 or both. Aminoacid substitutions of avian and environmental strains from Shanghai were obtained from reference 35. The appendix shows details of sequences deposited into GISAID database. Only strains with full-length sequence at the time of writing were included. Duplicate strain names but different sequences were excluded from the analysis. ND=not detected. *Gln226Ile was present in A/Hangzhou/1/2013(H7N9).

Table 2: **Key genetic mutations in individual viral proteins of 2013 H7N9 and 1997 H5N1 influenza viruses**

Previous exposure to other influenza viruses might also lead to rapid increase in non-neutralising antibodies against these viruses, which can be associated with more severe H7N9 disease.[83,84] Notably, most fatal or severe cases of 2013 H7N9 have comorbidities, and up to a quarter of adult patients were smokers.[15] The effect of genetic predisposition (eg, the *IFITM3* and CD55 polymorphisms) on H7N9 infection warrants further investigation.[85,86]

A higher percentage of elderly patients aged 65 years or older and a higher proportion of males were infected during the 2013 H7N9 than were in the 1997 H5N1 outbreak. One explanation for this finding could be that most grandmothers in Shanghai take care of their grandchildren at home while the retired grandfathers go to live poultry markets for grocery shopping every day.[87] Another unique feature is that no poultry workers were infected with H5N1 in the Hong Kong epidemic. Sero-epidemiology might ascertain the cause of this difference.

Human beings probably acquired the H7N9 virus via direct contact with infected poultry or their surroundings, although aerosol transmission between ferrets was reported.[77,88] A study of the 2003 H7N7 outbreak estimated

that 18% of transmission between farms was related to wind.[89] Up to May 22, 2013, 899758 animal and environmental samples had been tested. 53 samples were positive for the 2013 H7N9 virus; 51 of these were collected from poultry or environmental samples in poultry markets, one sample was collected from a wild pigeon in Nanjing, and one sample was collected from a domestic racing pigeon at a household farm in Nantong.[28] 2013 H7N9 viruses with very similar gene sequences have been found in pigeons, chickens, ducks, and environmental samples from poultry markets in affected areas.[11,35,36,44] As in the H5N1 outbreak in Hong Kong, most confirmed cases of H7N9 (55·9%) had history of contact with poultry.[15] Although some patients had contact with pigs, 2013 H7N9 has not been isolated from pigs.[14,36] In the 2013 outbreak, the H7N9 virus might have originated from poultry farms and was amplified in overcrowded live poultry markets before its transmission to human beings. Slaughtering and preparation of infected poultry constitute the highest risks of exposure to infected poultry secretions and excreta. Although transmission to human beings through contact with wild birds or consumption of inadequately cooked poultry is possible, documentation of such occurrences is scarce even though H7 viruses are able to survive and remain infective for a prolonged period.[90]

No definitive evidence of continuing person-to-person transmission exists, although some first-generation transmission might have occurred in four case clusters of which three were familial clusters.[14,91] However, exposure to a common avian or environmental source could not be excluded in these clusters. Few reports of person-to-person transmission of H5N1 have been recorded.[92] The predilection of the 2013 H7N9 virus for the lower respiratory tract—shown by the higher virus burden in patients sputum than throat swab—might explain the low number of person-to-person transmissions as in the case of H5N1.

132 reported H7N9 infections in patients from March to May 2013 has far exceeded the 45 reported H5N1 infections in China within the past 10 years.[61] The crude case fatality of 2013 H7N9 infection is 30%, which is lower than that for H5N1 infection (60%), but much higher than the 0·1% of the 2009 pandemic or seasonal influenza.[3,19] The true case fatality rate is uncertain because many of the patients are still receiving care in the intensive care units. Enhanced surveillance between March 7 and April 28, 2013, in which 20739 patients with influenza-like illness were tested, identified only two patients with H7N9 who were not hospitalised.[79]

Geographical location

The first human cases of H7N9 were detected in Shanghai, which is served by the farming and industrial establishments along the Yangtze River delta. Shanghai, a cosmopolitan megacity hardest hit by this novel virus, is similar to Hong Kong, a region that is served by the Pearl River delta and where the first human cases of H5N1 were detected in 1997.[1,75,76] Both cities have well established health-care infrastructure with the necessary diagnostic methods for the detection and characterisation of new viruses. Furthermore, both cities are located along the Asian-Australasian flyway, where migratory birds stop at their wetlands and have surrounding poultry farms serving the densely populated areas that have many live poultry markets (table 4).

For more on the **Asian-Australasian flyway** see http://www.eaaflyway.net/index.php

Between 1985 and 2011, consumption of poultry per person has increased 3·3 times from 3·2 kg to 10·6 kg in urban areas, whereas overall consumption of poultry increased 8·8 times from 1·53 to 13·54 million tonnes in China between 1987 and 2012.[93–95] Additionally, of all municipal areas or provinces in China, Shanghai has the highest densities of both people and poultry.[94] This is similar to the situation in Hong Kong, where poultry consumption increased greatly before the 1997 H5N1 outbreak (figure 4).[75,93,96] The emergence of the H7N9 virus in 2013 might also be related to the selective pressure induced by the widespread use of the H5N1 vaccine because H5 and H7 are the common subtypes that generally cause poultry outbreaks. China uses more than 90% of the H5N1 vaccine worldwide.[97] Although the H5 vaccine effectively suppresses the circulating clade of H5 virus in China, the vaccine cannot stop the emergence of antigenically drifted H5 and non-H5 viruses while biosecurity measures at wet markets and farms are suboptimum. Poor biosecurity allows the ready intrusion and amplification of a new virus reassortant. The source and mechanism of spread of the 2013 H7N9 outbreak

	Country	Details
H7N2		
2002[57]	USA	A culler of poultry had upper respiratory tract symptoms and was later found to have antibody against H7N2; outbreak in poultry occurred before the human case
2003[5]	USA	A man with HIV with conjunctivitis and community acquired pneumonia; no known exposure to live or dead poultry, wild birds, or bird faeces
2007[58]	UK	Four human infections presented with influenza-like illness; outbreak in poultry occurred before the human cases
H7N3		
2003[59]	Italy	3·8% of the poultry workers were positive for anti-H7 antibody
2004[7]	Canada	55 suspected and two laboratory-confirmed human infections; an outbreak in chicken arose before the human cases; both confirmed patients developed conjunctivitis
2006[60]	UK	A poultry worker presented with conjunctivitis. An outbreak in poultry arose before the human case
2012[61]	Mexico	Two human infections presented with conjunctivitis; preceded by poultry outbreak
H7N7		
1980[62]	USA	The virus was transmitted from an infected seal to a laboratory worker, who developed conjunctivitis
1996[63]	UK	One patient presented with conjunctivitis; while cleaning out her duck house, a piece of straw entered her eye; no preceding outbreak in the ducks
2003[9]	Netherlands	89 human infections with one fatality; an outbreak in chicken arose before the human cases

Table 3: **Reported human infections caused by H7 viruses before the 2013 influenza A H7N9 outbreak**

	2013 influenza A H7N9	1997 influenza A H5N1
Date and place of first case in a human being	February 2013, Shanghai	May 1997, Hong Kong
Wetland near the city	Chongming Dongtan nature reserve	Mai Po nature reserve
Human population at the time of outbreak	23·7 million people (permanent resident population)[75]	6·5 million people[76]
Subsequent spread to other geographical areas	Shanghai, Anhui, Zhejiang, Jiangsu, Beijing, Henan, Shandong, Jiangxi, Fujian, and Taiwan	China (including Hong Kong), southeast Asia, Middle East, and Africa*
Amplification host	Domestic chickens, pigeons, and ducks	Domestic chickens, ducks, and geese
Epidemic centres and % of infected poultry	Wet markets: farms (20% of chickens and 40% of pigeons at epidemiologically linked wet market infected)[11] but overall 0·006% for large-scale animal surveillance	Farms: wet markets (20% of chickens, 2% of ducks and geese infected)[29]
At-risk groups	Visitors to wet markets, backyard farm residents, and occupational related poultry handlers	Visitors to wet markets
History of poultry exposure	62 of 111 (55·9%)[15]	9 of 14 (64·0%)[77]
Mode of transmission	Poultry-to-human, little person-to-person transmission	Poultry-to-human, little person-to-person transmission
Preceding large poultry outbreak	No	Yes (three farms affected before first human case in March and April 1997)
Interval between first documented case and subsequent cases	9 days	6 months
Admission to intensive care unit	85 of 111 (76·6%)[15]	10 of 18 (55·6%)
Patient characteristics		
Age in years, median (range)†	61 (2–91)	9·5 (1–60)[74]
Age ≥65 years	54 of 128 (42%)	0 of 18 (0%)[74]
Age <18 years	6 of 128 (5%)	11 of 18 (61%)[74]
Male: female†	90:38	8:10[78]
Underlying diseases		
No underlying diseases	43 of 111 (38·7%)[15]	12 of 18 (66%)[74]
At-risk group‡	40 of 46 (87%)[14]	7 of 18 (39%)[74]
Median incubation period	5 days[15]	4 days
Crude case-fatality proportion†	40 of 132 (30%)	6 of 18 (33%)[74]

*The clade 0 of influenza A H5N1 virus was not found after 1997. The H5N1 virus infecting human beings since 2003 belongs to other clades. † On the basis of data from 132 symptomatic patients as of June 26, 2013. The age of four cases (cases 73–76) and the sex of four cases (cases 73–75, 77) were not reported. ‡ At-risk group based on extremes of age and underlying conditions as defined in reference 14; 27 of 111 cases of H7N9 were smokers.[18]

Table 4: **Epidemiological and clinical differences between human 2013 influenza A H7N9 and 1997 influenza A H5N1 infections**

between different geographical regions are still uncertain. The roles of movement of migratory birds, human beings, and poultry deserves further investigation.

Clinical features

In the H7N9 and H5N1 outbreaks, infected individuals presented with acute severe community-acquired pneumonia that did not respond to typical and atypical antimicrobial coverage. In an analysis of 111 of 132 reported cases of human H7N9 infections in 2013, more than 90% of patients had fever or cough, and 24% of the 111 patients had haemoptysis.[15] 71% of the patients had acute respiratory distress syndrome. Other complications associated with H7N9 infection are rhabdomyolysis, acute kidney injury, encephalopathy, and multiorgan dysfunction.[11,15] Unlike the 1997 H5N1 outbreak, in which nearly 40% of patients had only fever and upper respiratory tract symptoms, none of the 111 patients with 2013 H7N9 infection had sore throat, rhinorrhoea, or conjunctivitis, although mild rhinorrhoea was reported in a 3·5-year-old boy.[80] Gastrointestinal symptoms (13·5%) were less common in people infected with H7N9 than in those with the 1997 H5N1 infection (50%).[1,15] Reye's syndrome was reported in a child with 1997 H5N1 infection.[1] Gastrointestinal haemorrhage, reactive haemophagocytosis, and haemorrhagic pleural effusion, which were present in some cases of 1997 H5N1, have not been reported for H7N9 infection. Coinfection at presentation or during admission to hospital can occur, as reported for H5N1.[2,15,98]

Laboratory abnormalities in patients with either infections were prominent lymphopenia, thrombocytopenia, coagulopathy, and raised serum transaminase, creatine kinase, and C-reactive protein concentrations. Radiological features were similar in both infections: pulmonary consolidation (which progressed to involve bilateral lung fields), diffuse ground glass opacities, pleural effusions, and mediastinal emphysema. None of these clinical, laboratory, and radiological manifestations are pathognomonic for H7N9 infections. The definitive diagnosis depends on viral culture or real-time-PCR (RT-PCR) for the H7 and N9 gene targets from respiratory tract secretions. Because of the predilection of this virus for the lower respiratory tract, if available, sputum, endotracheal aspirate or bronchoalveolar lavage might give a better sensitivity than throat or nasopharyngeal specimens. However, point-of-care rapid antigen tests and multiplex RT-PCR assays have low sensitivity.[99] Acute and convalescent serum antibody testing by haemagglutination inhibition or neutralisation can be useful for epidemiology or retrospective diagnosis. Any febrile patients with history of travel to affected areas, or contact with poultry or infected patients can be tested for H7N9 as a screening strategy for the early identification of imported cases.

2013 H7N9 is probably resistant to adamantanes because of the presence of an M2 Ser31Asn mutation (table 2).[12] Both genetic analysis and in-vitro testing showed that most strains of 2013 H7N9 are susceptible to neuraminidase inhibitors.[100] However, two reported strains carry the neuraminidase Arg292Lys (N2 numbering) mutation, which is associated with resistance to oseltamivir and zanamivir (increase in half maximal inhibitory concentration [IC_{50}] more than 9000-fold for oseltamivir and between four-fold and 25-fold for zanamivir).[101] Although a delay in antiviral

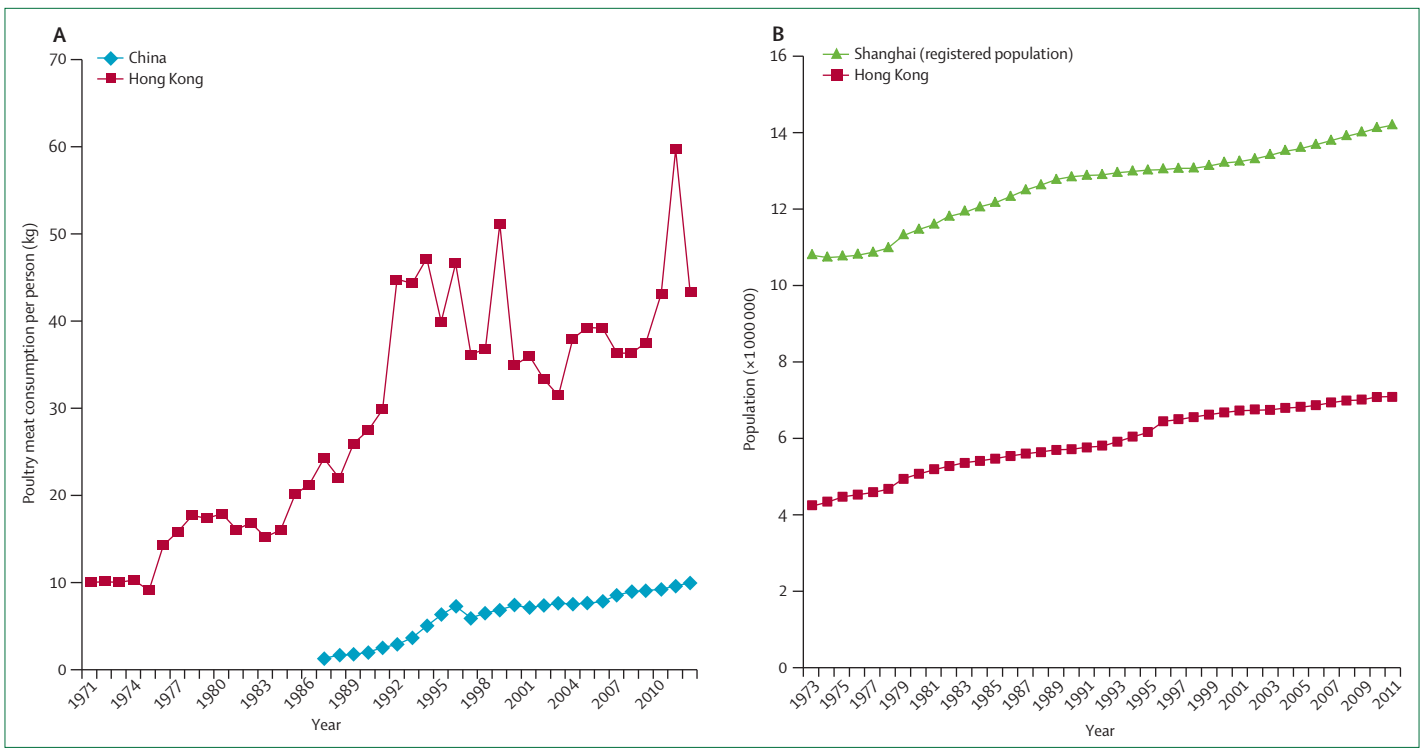

***Figure 4*: Comparison of poultry meat consumption between mainland China and Hong Kong and human population between Shanghai and Hong Kong**
(A) Poultry meat consumption in Hong Kong peaked just before 1997 but markedly decreased after the H5N1 outbreak. Poultry meat consumption in China is catching up. (B) Similar human population growth rates in Shanghai and Hong Kong. Data of poultry consumption for China are not available before 1987. Data from references 75, 93, and 96.

therapy of more than 3 days after symptom onset was not an independent risk factor for acute respiratory distress syndrome in 2013 H7N9 infections,[15] previous experience from H5N1, 2009 pandemic, and seasonal influenza infections suggest that early oseltamivir treatment started less than 2 days after symptom onset can reduce morbidity and mortality.[19,102] Inhalational neuraminidase inhibitors such as zanamivir and laninamivir are unlikely to be helpful in patients with respiratory failure.[103,104]

Because oseltamivir might not affect outcomes in patients with late and severe influenza pneumonia, intravenous zanamivir and peramivir should also be investigated in future treatment trials.[105] Other investigational antivirals include those acting on the haemagglutinin receptor (DAS181), nucleoprotein (nucleozin), polymerase (viramidine and T705), and protease (aprotinin).[106] Antivirals targeting the host machinery including nitazoxanide were also reported.[106–108] Although 2013 H7N9 is susceptible to neuraminidase inhibitors, the virus might develop resistance during treatment.[19] Mice infected with the influenza virus resistant to amantadine had improved survival when treated with the triple combination of amantadine, oseltamivir, and ribavirin.[109] Empirical antibiotics are initially needed to cover other common pathogens, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, but should be stopped when bacterial tests are negative.

The role of immunomodulation remains controversial. Glucocorticoids have been used in many patients with 2013 H7N9, but the benefit is unclear.[12,15] Non-steroidal anti-inflammatory drugs such as celecoxib improved survival of mice with H5N1 infection.[110] Conflicting outcomes of patients treated with statins and macrolides have been reported.[111] The plasma of convalescent patients, which contains specific neutralising antibodies, has been used in patients with the H5N1 virus, 1918 pandemic H1N1, and the 2009 pandemic H1N1. Hyperimmune globulin was also used in the 2009 pandemic. Both treatments have reduced morbidity and mortality in small studies.[112–115] Supportive measures in intensive care are especially important for patients with respiratory or multiorgan failure. Extracorporeal membrane oxygenation could be beneficial in patients with respiratory failure despite maximum mechanical ventilatory support.[116]

Control of poultry-to-human transmission

In view of the many similarities between the Hong Kong and Shanghai outbreaks, many of the measures implemented in Hong Kong can be, and have already been, applied to the current outbreak in eastern China. In the 1997 Hong Kong outbreak, temporary closure of live poultry markets and cessation of poultry trading were instituted. Subsequently, different poultry species were segregated to reduce the risk of further genetic

reassortment. The regular cleansing of designated transport cages in live poultry markets was introduced to stop the virus trafficking between farms and markets. To interrupt the amplification of influenza virus, a monthly rest day with no live poultry allowed in the wet market was implemented in Hong Kong in July 2001. The isolation rate of H9N2 from poultry in live markets before the rest day could be as high as 10%, but reduced to less than 1% after the rest day.[117] To further reduce the spread of influenza virus in wet markets, overnight poultry storage was banned in July 2008.[118] A further 84% reduction in the isolation rate of H9N2 was reported. As in the H5N1 outbreak, poultry vaccination specifically against the H7N9 virus could probably reduce its transmission. A mathematical model showed that poultry vaccination with a matched H5N1 vaccine with coverage of only 30% of the poultry reduces the basic reproductive number to less than one.[117]

The 1997 H5N1 human outbreak was stopped after a cull of all poultry in Hong Kong.[117] Subsequently, only registered farms with stringent biosecurity measures in China can import chickens to Hong Kong. If a highly pathogenic avian influenza virus outbreak arose, all live poultry and poultry products from the affected province would be suspended for up to 21 days. For unaffected farms within 3 km of the index farm affected by the highly pathogenic avian influenza virus, there would be a 90 day suspension for live poultry and poultry products. For farms where 2013 H7N9 is detected by virological testing, similar depopulation and perimetric moratorium strategies of poultry control are necessary to control the source.

The general public was educated to avoid contact with birds or poultry, to cook poultry thoroughly, and to comply with hand hygiene because consumption of raw poultry might be a route of transmission. Public education and administrative measures have also led to a slow change in eating habits from live chicken to chilled or frozen chicken. To reduce the contact between human beings and poultry in Hong Kong, the Hong Kong Government implemented a voluntary surrender scheme in 2004, and subsequently a buyout scheme in 2008 for poultry retailers, wholesalers, transporters, and farmers. In 2006, domestic households were prohibited to keep any live poultry. With these measures, between 1997 and 2013, the number of local poultry farms in Hong Kong decreased from about 800 to 30 and the number of retail poultry stalls decreased from about 800 to 131. Since 1997, the number of imported live chickens was reduced from about 100 000 per day to 7000 per day. Enhanced surveillance of H5N1 and H7N9 in both live and dead wild birds, farm, and market poultries might be important for geographical areas along the migratory route of wild birds. With these measures, no local cases of H5N1 in Hong Kong have been identified since 2007, despite the failure to implement central slaughtering because of cultural resistance.

Is 2013 H7N9 a concern?

The 2013 H7N9 outbreak in human beings resembles the 1997 H5N1 outbreak in many ways, including substantial mortality associated with severe pneumonia, multiorgan dysfunction, and cytokine dysregulation, appearance in cities along the route of migratory birds with high density of poultry, predominant poultry-to-human transmission, and the predilection for the lower respiratory tract.[11,119] The high proportion of elderly men infected with H7N9 could be attributable to different social circumstances leading to this age and sex bias. The rapid increase in human beings infected with severe H7N9 to more than 130 cases within 2 months from March to May, 2013, is unprecedented for avian influenza viruses and could be related to enhanced transmissibility from poultry to human or improved virological testing. However, H7N9 is difficult to control because flock die-off did not precede human cases. Stringent measures to control the poultry outbreak will stop the human epidemic and perhaps decrease the risk of H7N9 evolving to become a pandemic agent.

Even though this dangerous scenario has not happened with H5N1, the virus has spread from Hong Kong to the whole of China, southeast Asia, Middle East, and Africa.[2] Although the number of human beings infected with H7N9 was greatly reduced in May 2013 (possibly related to the rising ambient temperature or the epidemiological control measures in live poultry markets), high vigilance is still needed in view of the interval of 6 months between the first and second 1997 H5N1 human case. Biosecurity measures for live poultry markets, farms, and trafficking should be commensurate with increasing demand for poultry. Transmission cycles within markets or between markets and farms could be interrupted by a no overnight poultry policy with daily poultry-stall cleansing and ultimately central slaughtering in cities.

Precautionary research and development of poultry and human H7N9 vaccines are important. Control in rural areas with many backyard farms relies on poultry vaccination. Virological surveillance of wild birds and domestic poultry in farms and markets is important in understanding the emergence of the 2013 H7N9 and other avian influenza viruses. Over 30 years from 1981 to 2011, the consumption of poultry in Hong Kong has tripled to 60 kg per person per year. In 2012, the consumption of poultry in China reached 10 kg per person per year, and the mainland cities such as Shanghai are likely to catch up with the amount of consumption in Hong Kong in coming years (figure 4).[95] Increasing problems with avian influenza in poultry and human beings are anticipated.

Conflicts of interest
We declare that we have no conflicts of interest.

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Contributors

KKWT, JFWC, HC, LL, and K-YY did the literature search, data analysis, and drafted the manuscript. All authors have seen and approved the final version

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