Rev. sci. tech. Off. int. Epiz., 2009, **28** (1), 39-47

Molecular epidemiology of H5N1 avian influenza

Y. Guan [(1)], G.J.D. Smith [(1)], R. Webby [(2)] & R.G. Webster [(2)]

(1) State Key Laboratory of Emerging Infectious Diseases, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China
(2) Virology Division, Department of Infectious Diseases, St Jude Children's Research Hospital, Memphis, TN 38105, United States of America

Summary
The highly pathogenic Asian H5N1 influenza virus that was first detected in Guangdong in the People's Republic of China (China) in 1996 is unique in having spread to humans and other mammalian species. To date, this virus has not consistently transmitted between any mammalian species but the continued spread and evolution of these viruses in domestic poultry across Eurasia presents a continuing pandemic threat. These viruses have caused devastation in domestic poultry and have killed over 60% of infected humans. The H5N1 viruses are unique in having evolved into multiple clades and subclades by reassortment with other influenza viruses in the epicentre of southern China, and accumulation of point mutations has resulted in antigenic differences between the clades. Three waves of spread have occurred, wave one to East Asia and Southeast Asia, wave two through Qinghai Lake, China, to Europe, India and Africa, and wave three to Southeast Asia again. This paper deals with the molecular epidemiology of the evolution of the multiplicity of H5N1 clades. The continuing evolution of these H5N1 viruses and the possible establishment of secondary epicentres in Indonesia, Egypt and Nigeria present a continuing threat to poultry and people globally.

Keywords
Avian influenza – Dissemination – H5N1 – Highly pathogenic avian influenza – Molecular evolution – Pandemic potential.

Introduction

Highly pathogenic avian influenza viruses (HPAIV) of subtype H5N1 have become panzootic in many Asian and African countries, resulting in repeated outbreaks in poultry and increased cases of human infection, giving rise to a persistent pandemic threat. In this paper the authors summarise the evolution and development of this H5N1 virus lineage.

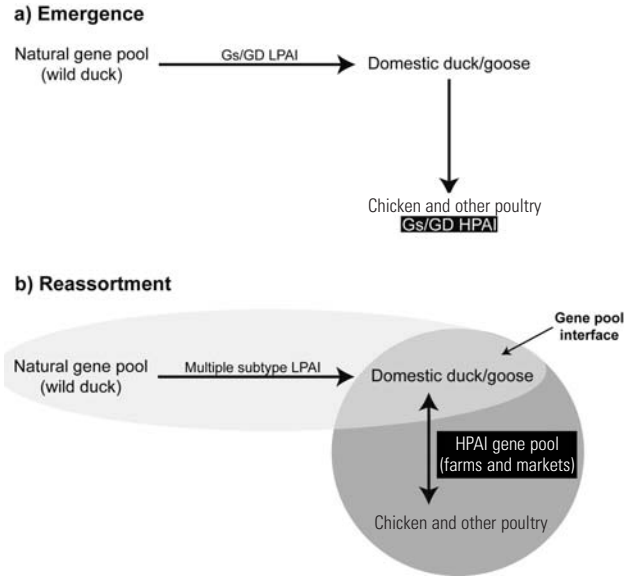
The first outbreak of the Asian H5N1 virus lineage was observed in geese in Guangdong in the People's Republic of China (China), in 1996 (31). Subsequently, a reassortant variant derived from Goose/Guangdong/1/96 virus (Gs/GD/1/96) caused the 'Bird Flu' incident in Hong Kong in 1997 and demonstrated that a 'pure' avian influenza virus could be directly introduced to humans and cause infection (8, 25, 31). This demonstrated a possible pathway for the generation of a new pandemic influenza (25).

Genetic analyses suggested that the H5N1 virus causing human infection in Hong Kong, represented by A/Hong Kong/156/97 (HK/97-like), may have been generated by multiple reassortment events between Gs/GD-like and H9N2 or H6N1 viruses found in terrestrial poultry (15, 16, 31). Following the 1997 outbreak, HPAI H5N1 variants with Gs/GD-like haemagglutinin (HA) and neuraminidase (NA) genes became endemic in poultry in southern China (12, 13). Those variants arose through a series of reassortment events to incorporate different gene segments with different origins. Eventually, these H5N1 viruses evolved into genetic and antigenic sublineages or clades

that allow us to trace their transmission routes and unveil the evolutionary events behind their genesis.

The origin of Goose/Guangdong/1/1996

Phylogenetic analyses of low pathogenic avian influenza (LPAI) H5 subtype viruses, isolated from poultry and migratory birds in Eurasia, revealed that four gene segments of Gs/GD/1/96 were closely related to viruses detected from migratory birds in Hokkaido, Japan (10). Dating the emergence of each gene segment of Gs/GD/1/96 further demonstrated that these gene segments had a very similar time of emergence in poultry in southern China (26). These findings suggested that the prototype virus was introduced into poultry as a non-reassortant LPAI H5N1 virus from wild bird populations (Fig. 1).



LPAI: low pathogenic avian influenza
HPAI: highly pathogenic avian influenza

Fig. 1
The emergence of the A/Goose/Guangdong/1/96-like H5N1 viruses from the natural gene pool (a) and the interaction between the low pathogenic natural gene pool and the highly pathogenic gene pool in poultry (b)

Evolutionary patterns and development in China

More than 20 outbreaks of HPAIV H5 or H7 were recorded in the 20th Century (1). Genetic analyses and laboratory experiments suggested that all of those H5 and H7 viruses evolved from low pathogenic precursors, and developed their high pathogenicity after introduction into terrestrial poultry. However, the Gs/GD-like H5N1 virus lineage is the only example that is known to have generated multiple genetic reassortants, or genotypes. Furthermore, H5N1 viruses with different gene constellations have caused outbreak episodes in different regions and at different times (e.g. 9). To better understand the evolution and development of this virus lineage, the authors address this issue chronologically.

Initial H5N1 reassortant variants: HK/97, Hubei/97

The original outbreak of HK/97-like H5N1 virus occurred in April 1997 in several chicken farms in the New Territories of Hong Kong (Hong Kong can be divided into three areas for administrative purposes; the New Territories, which border Guangdong Province of China, is the largest of these areas) (21). This outbreak was associated with the first or index human case, a five-year old boy, of H5N1 influenza infection in May (8, 25). However, the major outbreak in local live poultry markets occurred from late November to December 1997. The virus may well have been in the live poultry markets for months since no surveillance was performed. An additional 17 human cases were identified during this period of time. Even though the outbreak was terminated by depopulation of poultry in Hong Kong, the source or origins of this H5N1 variant are still not fully understood.

Genetic analysis of the HK/97-like H5N1 influenza virus revealed that it was a double or triple reassortant: the HA gene was derived from the Gs/GD-like virus, while the remaining seven genes were derived from H9N2 (quail/Hong Kong/G1/97-like) and H6N1 (teal/Hong Kong/W312/97-like) viruses (15, 31). Since H5, H6 and H9 subtypes of influenza virus were prevalent simultaneously in the live poultry markets of Hong Kong, and given that the HK/97-like virus had never been isolated from any other place, it was reasonable to believe that this H5N1 variant was generated locally in Hong Kong poultry markets. However, a recent study has indicated that the W312-like H6N1 virus was not a precursor of the HK/97-like virus but rather the descendant of an unidentified reassortant between the HK/97-like H5N1 virus and another H6N1 virus (7). Also, recently released sequence data suggested that other H5N1 reassortants were also detected in Hubei Province in 1997 (Hubei/97-like virus) (9). Interestingly, those variants contained some internal genes that are not related to contemporary field viruses but rather are closely related to influenza viruses isolated in the 1970s in Eurasia (9, 10, 18). These findings, in conjunction with the continuing emergence of multiple novel H5N1 reassortants, suggest that highly pathogenic Asian H5N1 viruses were circulating in the region prior to 1997.

Second reassortment events: genotypes B, Z and X series

Even though HK/97-like H5N1 virus was not detected in the field after the Hong Kong 'Bird Flu' incident, the Gs/GD-like virus was still prevalent in the goose population in southern China, as it was regularly detected from geese imported into Hong Kong from 1999 to 2000 (12). Studies in apparently healthy ducks in southern China demonstrated that H5N1 viruses were circulating in the region from 1999 through 2002 (3). By mid-December 2000, novel reassortants of the Gs/GD-like H5N1 HPAI viruses were detected from both domestic ducks and geese in the authors' surveillance network (12, 28). Afterwards, the novel H5N1 reassortants gradually replaced the Gs/GD-like viruses in poultry (12, 14, 17).

During this new reassortment event multiple H5N1 variants were generated. These novel reassortant viruses (e.g. genotypes B, Z and X series) caused a new dissemination and outbreak wave in poultry in China. Although a major outbreak was first reported in live poultry markets in Hong Kong in May 2001, available sequence data suggested that the H5N1 HPAI outbreak also occurred elsewhere in China (9). In 2000, the Gs/GD lineage viruses were detected in five provinces (Fujian, Guangdong, Guangxi, Jiangsu and Zhejiang) of eastern and southern China along with novel genotypes (Table I). In 2001 eight provinces were affected by H5N1 and almost all isolates were novel reassortants (9).

In 2001, five different genotypes or reassortants were simultaneously identified during the Hong Kong outbreak in poultry (genotypes A to E) (13). This was a new situation in influenza epidemiology as each previously recorded HPAI outbreak had been associated with only a single genotype. Phylogenetic analysis of full genome sequences revealed that at least another five H5N1 reassortants were recognised from five provinces in 2000, and in the following year as many as fifteen reassortants, or genotypes, were recognised from eight provinces (Table I). One of the latter H5N1 viruses that became dominant, named genotype Z, was also first detected in Guangxi in 2001. The viruses were detected not only in the south-east coast region, but also in Hebei and Henan Provinces in central and north central China. It was also during this period that the first HPAI H5N1 viruses were detected outside of China, in Vietnam (3, 19, 29).

Continued emergence of novel genotypes and genotype replacement in China

There was a further expansion of H5N1 activity in China in 2002 (9). Viruses were detected in four additional provinces (Jilin, Hubei, Hunan and Yunnan) while the virus was still detected in all provinces affected in the previous year (Table I). Genetic and phylogenetic analyses of available sequence data recognised 15 distinct reassortants, or genotypes in 2002. Four genotypes (B, Z, W and X0) had already been detected in the previous year, but the remaining 11 genotypes emerged in 2002. Among those novel reassortants most belonged to the genotype X series (genotypes X1 to X9), with only four (genotypes B, W, Z and Z^+) persisting to the following year (Table I).

Table I
H5N1 influenza virus activity and genotype distribution in China, 1996 to 2006

Year	Province (genotype)
1996	Guangdong [(a)] (Gs/GD)
1997	Guangdong (Gs/GD, HK/97-like), Hubei (Hubei-like)
1999	Guangdong (Gs/GD)
2000	Fujian (F), Guangdong (Gs/GD, Gs/GD$^-$, C, T1), Guangxi (*ND**), Jiangsu (*ND*), Zhejiang (Gs/GD1, W)
2001	Fujian (B, W), Guangdong (Gs/GD$^-$, A-E, X0, X10, W1, B2, T3), Guangxi (B, B1, Z), Hebei (Hubei-like), Henan (*ND*), Jiangsu (*ND*), Shanghai (Gs/GD$^-$, C, X0), Zhejiang (*ND*)
2002	Fujian (X7), Guangdong (X0-X3, X9, B, B3, Z, Z^+, W), Guangxi (X6), Hebei (X8), Henan (ND), Hubei (ND), Hunan (Z), Jiangsu (B), Jilin (ND), Shanghai (X4, X5, Z), Yunnan (Z), Zhejiang (*ND*)
2003	Beijing (V), Fujian (B, W), Guangdong (Z, V), Guangxi (*ND*), Henan (*ND*), Hubei (V), Hunan (Z), Jiangsu (*ND*), Jilin (T4, Z), Shangdong (*ND*), Yunnan (Z)
2004	Anhui (*ND*), Fujian (Z^+), Guangdong (Z, V, V1, Hubei-like), Guangxi (Z, W, G, W2, Hubei-like), Henan (Z, Hubei-like), Hubei (Z), Hunan (Z, V), Jiangsu (*ND*), Jilin (T5), Shandong (B, X0, Hubei-like), Shanghai (Z), Yunnan (Z)
2005	Anhui (*ND*), Fujian (Z, V), Guangdong (Z, V, V1), Guangxi (Z, V, G), Guizhou (Z, G, V), Hebei (V), Hubei (*ND*), Hunan (Z, V, G, T6), Jiangsu (*ND*), Jiangxi (Z, V), Qinghai (Z), Yunnan (Z, V, G), Zhejiang (*ND*)
2006	Fujian (V), Guangdong (Z, V, G, V2, V4), Guangxi (V, G), Guizhou (G, V, V3), Hunan (Z, V), Jiangsu (*ND*), Qinghai (Z), Shanxi (V), Yunnan (V), Zhejiang (V)

(a) Includes Hong Kong Special Administrative Region and Shantou
* *ND* denotes genotype could not be determined as full genome is not available

Most of the H5N1 variants were identified based on the viruses associated with disease outbreaks at the live-poultry markets and farms in Hong Kong (21, 22). It was noted that most of the H5N1 isolates detected between January and March 2003 were genetically diverse and belonged to different genotypes; however, the majority were genotype Z viruses (9, 17). This suggests that genotype Z viruses gradually became dominant during 2002, while most of those novel reassortants were transient (Table I). After 2002, novel reassortant H5N1 viruses continued emerging almost every year in poultry in China. These included genotype V in 2003, and genotypes V1 and G in 2004 (9). From 2002 to 2004, H5N1 virus activity was continually observed in several of China's 22 provinces (in 12 provinces in 2002, 11 provinces in 2003, and 12 provinces in 2004). It is worth noting that genotype V virus has gradually replaced genotype Z since the emergence of the Fujian-like (clade 2.3.4) H5N1 variant in early 2005 (9, 23). By 2006, genotype V was detected in 8 out of 10 provinces where genotypes were defined, while genotypes Z and G were only detected in three provinces each (Table I).

Dating the emergence of each genotype of H5N1 viruses revealed that those reassortants were generated by three distinct reassortment events (26). The first event generated genotypes B and W, while the second one resulted in the emergence of the genotype X series. These two reassortment events probably occurred from early 2000 to mid-2001. The most recent variants, including genotypes Z, Z+, V, V1 and G, were generated by further interaction or reassortment between viruses from the first two reassortant groups (26). It is also apparent that reassortment between influenza viruses from domestic and migratory birds has contributed to the expanded diversity of the influenza virus gene pool among poultry in Eurasia (10). Specifically, different subtypes of avian influenza virus from the natural gene pool in wild birds are introduced into domestic ducks where these viruses undergo regular reassortment with endemic H5N1 viruses (Fig. 1). Subsequently, transmission of these reassortant viruses within large, highly connected populations of duck and other poultry species results in frequent interspecies transmission and genetic drift (26).

Prevalence and dissemination

Since it was first detected in 1996, Gs/GD-like H5N1 influenza virus and its variants have spread to over 60 countries across Eurasia and Africa. The viruses have become endemic and panzootic in some of these countries and have caused repeated poultry outbreaks and human infection, posing an unprecedented global pandemic threat. In this section, the authors will discuss how this virus has spread beyond China.

Endemicity in poultry in China

Prospective virological surveillance in live-poultry markets across southern China provided evidence for the endemicity of highly pathogenic H5N1 in the region. In the 18 months between January 2004 and June 2005, H5N1 virus was most frequently isolated from apparently healthy ducks and geese (isolation rates, 1.8% and 1.9%, respectively), followed by minor poultry (0.46%) and chickens (0.26%) (5, 23). Live poultry markets tested positive for H5N1 virus in 16 of the 18 months during this period (overall positivity rate was approximately 0.9%). From July 2005 to June 2006, H5N1 virus was detected in each month tested. The isolation rates for ducks, geese and chickens were 3.3%, 3.5% and 0.5%, respectively.

Description of haemagglutinin drift from Goose/Guangdong to present

While the Gs/GD-like H5N1 virus continually reassorted with other influenza viruses to produce many different reassortants, the HA gene had continually accumulated point mutations that gave rise to significant antigenic drift. The observed antigenic drift enables us to trace the transmission pathways of H5N1 viruses and also created additional challenges in selecting vaccine candidates for pandemic preparedness.

Initially, surveillance in southern China showed that H5N1 influenza viruses isolated from different provinces were antigenically and genetically distinct. In particular, viruses from Guangdong, Guiyang, Hunan and Yunnan grouped together, indicating isolated evolution within these regions (5). The same situation was also observed for viruses from Vietnam and Indonesia (5, 24). However, there were also a number of HA sublineages from China that contained viruses isolated from different provinces that reflected the interaction of viruses from different regions. Genetic and antigenic analyses of viruses isolated from different regions or countries revealed that multiple distinct sublineages had become established in several regions where the H5N1 variants had been endemic for several years. These sublineages, subsequently incorporated into the World Health Organization (WHO) H5N1 nomenclature system as different clades, include the viruses from Vietnam/Thailand and Malaysia (clade 1), from Indonesia (clade 2.1), and the most recently recognised clade 2.3.4 viruses first described as 'Fujian-like' (23, 30). The current known geographical distribution of different H5N1 HA clades, as defined by the WHO/World Organisation for Animal Health/Food and Agriculture Organization H5N1 Evolution Working Group (30), is provided in Figure 2. Since a human pandemic virus could possibly emerge from any country where H5N1 viruses are endemic, it has been necessary for the WHO to include viruses from seven clades as pre-pandemic vaccine candidates to ensure adequate antigenic coverage of circulating H5N1 viruses.

Fig. 2
Map showing country distribution of A/Goose/Guangdong/1/96-like H5N1 virus haemagglutinin clades reported from poultry and wild birds since 1996

Transmission to East Asia and Southeast Asia (wave 1)

As many as eight eastern and southeastern Asian countries recorded, almost simultaneously, H5N1 HPAI outbreaks from November 2003 to February 2004, constituting the first H5N1 outbreak and transmission wave (17). All of the viruses detected in these countries during wave 1 were genotype Z. Even though the direct precursors of the viruses detected in South Korea and Japan have not been identified, genetic and antigenic analyses of isolates from different provinces of southern China revealed a similar virus isolated from chickens in Guangdong in early 2003 (5). The viruses prevailing in Vietnam, Thailand, Laos, Cambodia and Malaysia (clade 1) were derived from Yunnan province of China, with which Laos and Vietnam share a land border (Fig. 2), while the viruses prevailing in Indonesia were derived from the viruses from Hunan isolated in early 2003 (5, 17, 23, 24, 27). Thus, available information suggests that all H5N1 variants from the first transmission and outbreak wave were introduced from China, most probably via movement of poultry or poultry products.

Transmission to Central Asia and Europe, and the development of endemicity in Africa (wave 2)

The second major transmission and outbreak wave of H5N1 virus was initiated following the Qinghai Lake outbreak in migratory birds in April 2005 (4, 6). Despite continued endemicity of HPAIV H5N1 in China over many years, the Qinghai outbreak (Qinghai is in western China) produced the first observed large-scale death of migratory birds. The affected bird species included bar-headed geese, great black-headed gulls and brown-headed gulls. Afterwards, viruses closely related to those isolated from the Qinghai Lake outbreak (QH-like virus, clade 2.2) were detected in other countries to the west and northwest (Fig. 2). Within seven months, clade 2.2 viruses were transmitted from China to Mongolia, Siberia, Central Asia, the Middle East, eastern and western Europe, and were eventually introduced into Africa (11).

Unlike the viruses detected in China, all isolates detected in this transmission and outbreak wave were genetically and antigenically very similar, although more marked

antigenic drift was observed in African countries after the virus had been maintained for several years. Since virus spread occurred so rapidly, it was believed that interaction of poultry and migratory birds was responsible for virus transmission. While the specific wild bird species involved are not known, accumulated information suggests that many different birds are permissive for the H5N1 HPAIV (e.g. 2), and that some of them may survive H5N1 infection. The question remains whether other descendants of these H5N1 viruses will continue to evolve and spread from secondary sites.

Renewed transmission to Southeast Asia (wave 3)

In 2006, updated virological and epidemiological findings from live-bird market surveillance in southern China demonstrated that H5N1 influenza viruses continued to be panzootic in different species of poultry (23). Genetic and antigenic analyses revealed the emergence and predominance of a previously uncharacterised H5N1 virus sublineage. The index variant was first isolated from ducks in Fujian in March 2005. By late 2005, about half of H5N1 influenza isolates from surveillance in southern China were Fujian-like strains. Since early 2006, the majority of H5N1 influenza isolates from southern China have belonged to this novel sublineage. This virus was also transmitted to Hong Kong, Laos, Malaysia, Thailand and Vietnam (23). The variant was also responsible for all human infections that were reported from late 2005 onwards. Genetic analysis revealed that this variant was a new genotype (V) which had a novel PA gene compared to the previously dominant genotype Z (9).

Recent epidemiological findings suggested that in northern Vietnam this variant (genotype V, clade 2.3.4) also replaced the previously endemic strains, but in southern Vietnam the viruses still belonged to clade 1 (20). Therefore, available information indicates that while genotype Z is still predominant in Indonesia, much of Southeast Asia, and those areas affected by clade 2.2 viruses, it appears that genotype V associated with the emergence of clade 2.3.4 viruses is predominant in much of China and northern Vietnam. Given the previous patterns of dissemination, it is also possible that clade 2.3.4 viruses may become established in other currently affected Southeast Asian countries. Furthermore, H5N1 virus endemicity in poultry across such a vast geographical area will continue to drive genetic and antigenic change until virus populations can be effectively brought under control.

Acknowledgements

This study was supported by the National Institutes of Health in the United States of America (National Institute of Allergy and Infectious Diseases [NIAID] contract HHSN266200700005C), the American Lebanese Syrian Associated Charities, and by the Area Excellence Scheme of the University Grants Committee (grant AoE/M-12/6) and the Research Grants Council (HKU 7512/06M) of the Hong Kong Special Administrative Region Government. G.J.D. Smith is supported by a career development award under NIAID contract HHSN266200700005C.

Épidémiologie moléculaire du sous-type H5N1 du virus de l'influenza aviaire

Y. Guan, G.J.D. Smith, R. Webby & R.G. Webster

Résumé

Le virus asiatique H5N1 de l'influenza aviaire hautement pathogène, détecté pour la première fois en 1996 à Guangdong en République populaire de Chine, présente la caractéristique unique de s'être transmis à l'homme et à d'autres espèces de mammifères. Jusqu'à présent, il n'a pas été constaté de transmission régulière entre espèces chez les mammifères ; en revanche, la propagation continue et l'évolution de ces virus parmi les volailles domestiques à travers tout le continent eurasien fait planer une menace permanente de pandémie. Ces virus ont eu des effets dévastateurs chez les volailles domestiques ; chez les humains infectés, le taux de mortalité dépasse 60 %. Les virus de type H5N1 ont pour caractéristique unique d'avoir évolué en plusieurs clades et sous-clades suite au réassortiment avec d'autres virus de l'influenza dans l'épicentre situé en Chine méridionale ; l'accumulation de mutations ponctuelles s'est finalement traduite par une différenciation des caractères antigéniques de ces clades. Trois vagues épidémiques se sont succédé : la première a touché l'Asie de l'Est et du Sud-Est, la deuxième a traversé le lac de Qinghai en Chine pour gagner l'Europe, l'Inde et l'Afrique, tandis que la troisième a de nouveau affecté l'Asie du Sud-Est. Cet article est consacré à l'épidémiologie moléculaire de l'évolution des multiples clades des virus H5N1. L'évolution continue de ces virus H5N1 et le risque d'établissement d'épicentres secondaires en Indonésie, en Égypte et au Nigeria représentent une menace permanente et mondiale pour les populations aviaires ainsi que pour l'homme.

Mots-clés

Dissémination – Évolution moléculaire – H5N1 – Influenza aviaire – Influenza aviaire hautement pathogène – Pathogénie – Risque de pandémie.

■

Epidemiología molecular del subtipo H5N1 del virus de la influenza aviar

Y. Guan, G.J.D. Smith, R. Webby & R.G. Webster

Resumen

El subtipo H5N1 del virus de la influenza aviar altamente patógena, detectado por primera vez en 1996 en Guangdong (La República Popular de China), es el único que se ha propagado a seres humanos y otras especies de mamíferos. Hasta la fecha, no se ha transmitido sistemáticamente entre especies de mamíferos, pero su difusión y evolución constantes en las aves de corral de toda Eurasia constituyen una amenaza permanente de pandemia. El subtipo H5N1, que causó estragos en las aves de corral y provocó la muerte de más del 60% de los seres humanos infectados, es el único que dio lugar a la aparición de múltiples clados

y subclados debido a la recombinación con otros virus de la influenza aviar en el epicentro del Sur de China; además, la acumulación de mutaciones originó diferencias antigénicas entre los clados. Se han registrado tres olas de propagación. La primera hacia el Este y Sudeste de Asia; la segunda desde el lago Qinghai, China, hacia Europa, India y África, y la tercera hacia el Sudeste asiático nuevamente. En este artículo se examina la epidemiología molecular de la evolución de los numerosos clados del H5N1. Su evolución permanente y la posible aparición de epicentros secundarios en Indonesia, Egipto y Nigeria, amenazan constantemente a las aves de corral y los seres humanos en todo el mundo.

Palabras clave
Evolución molecular – H5N1 – Influenza aviar – Influenza aviar altamente patógena – Potencial pandémico – Propagación.

References

1. Alexander D.J. (2007). – An overview of the epidemiology of avian influenza. *Vaccine*, **25**, 5637-5644.

2. Brown J.D., Stallknecht D.E. & Swayne D.E. (2008). – Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. *Emerg. infect. Dis.*, **14**, 136-142.

3. Chen H., Deng G., Li Z., Tian G., Jiao P., Zhang L., Liu Z., Webster R.G. & Yu K. (2004). – The evolution of H5N1 influenza viruses in ducks in southern China. *Proc. natl Acad. Sci. USA*, **101**, 10452-10457.

4. Chen H., Li Y., Li Z., Shi J., Shinya K., Deng G., Qi Q., Tian G. *et al.* (2006). – Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in Western China. *J. Virol.*, **80**, 5976-5983.

5. Chen H., Smith G.J.D., Li K.S., Wang J., Fan X.H., Rayner J.M., Vijaykrishna D., Zhang J.X. *et al.* (2006). – Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc. natl Acad. Sci. USA*, **103**, 2845-2850.

6. Chen H., Smith G.J.D., Zhang S.Y., Qin K., Wang J., Li K.S., Webster R.G., Peiris J.S.M. & Guan Y. (2005). – H5N1 virus outbreak in migratory waterfowl. *Nature*, **436**, 191-192.

7. Cheung C.L., Vijaykrishna D., Smith G.J.D., Fan X.H., Zhang J.X., Bahl J., Duan L., Huang K. *et al.* (2007). – Establishment of influenza A virus (H6N1) in minor poultry species in southern China. *J. Virol.*, **81**, 10402-10412.

8. De Jong J.C., Class E.C.J., Osterhaus A.D.M.E., Webster R.G. & Lim W.L. (1997). – A pandemic warning? *Nature*, **389**, 554.

9. Duan L., Bahl J., Smith G.J., Wang J., Vijaykrishna D., Zhang L.J., Zhang J.X., Li K.S. *et al.* (2008). – The development and genetic diversity of H5N1 influenza virus in China, 1996-2006. *Virology*, **380** (2), 243-254; doi: 10.1016/j.virolo.2008.07.038.

10. Duan L., Campitelli L., Fan X.H., Leung Y.H.C., Vijaykrishna D., Zhang J.X., Donatelli I., Delogu M. *et al.* (2007). – Characterization of low-pathogenic H5 subtype influenza viruses from Eurasia: implications for the origin of highly pathogenic H5N1 viruses. *J. Virol.*, **81**, 7529-7539.

11. Food and Agriculture Organization of the United Nations (FAO) (2008). – Avian Influenza Disease Emergence Bulletin. Available at: http://www.fao.org/avianflu/en/AIDEnews.html (accessed on 23 January 2009).

12. Guan Y., Peiris J.S.M., Kong K.F., Dyrting K.C., Ellis T.M., Sit T., Zhang L.J. & Shortridge K.F. (2002). – H5N1 influenza viruses isolated from geese in southeastern China: evidence for genetic reassortment and interspecies transmission to ducks. *Virology*, **292**, 16-23.

13. Guan Y., Peiris J.S.M., Lipatov A.S., Ellis T.M., Dyrting K.C., Krauss S., Zhang L.J., Webster R.G. & Shortridge K.F. (2002). – Emergence of multiple genotypes of H5N1 avian influenza viruses in Hong Kong SAR. *Proc. natl Acad. Sci. USA*, **99**, 8950-8955.

14. Guan Y., Poon L.L.M., Cheung C.Y., Ellis T.M., Lim W., Lipatov A.S., Chan K.H., Sturm-Ramirez K.M. *et al.* (2004). – H5N1 influenza: a protean pandemic threat. *Proc. natl Acad. Sci. USA*, **101**, 8156-8161.

15. Guan Y., Shortridge K.F., Krauss S. & Webster R.G. (1999). – Molecular characterization of H9N2 influenza viruses: were they the donors of the 'internal' genes of H5N1 viruses in Hong Kong? *Proc. natl Acad. Sci. USA*, **96**, 9363-9367.

16. Hoffmann E., Stech J., Leneva I., Krauss S., Scholtissek C., Chin P.S., Peiris M., Shortridge K.F. & Webster R.G. (2000). – Characterization of the influenza A virus gene pool in avian species in southern China: was H6N1 a derivative or a precursor of H5N1? *J. Virol.*, **74**, 6309-6315.

17. Li K.S., Guan Y., Wang J., Smith G.J.D., Xu K.M., Duan L., Rahardjo A.P., Puthavathana P. *et al.* (2004). – Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature*, **430**, 209-213.

18. Lu J.H., Liu X.F., Shao W.X., Liu Y.L., Wei D.P. & Liu H.Q. (2005). – Phylogenetic analysis of eight genes of H9N2 subtype influenza virus: a mainland China strain possessing early isolates' genes that have been circulating. *Virus Genes*, **31**, 163-169.

19. Nguyen D.C., Uyeki T.M., Jadhao S., Maines T., Shaw M., Matsuoka Y., Smith C., Rowe T. *et al.* (2005). – Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1, from poultry in live bird markets in Hanoi, Vietnam, in 2001. *J. Virol.*, **79**, 4201-4212.

20. Nguyen T.D., Nguyen T.V., Vijaykrishna D., Guan Y., Peiris J.S.M. & Smith G.J.D. (2008). – Multiple lineages of influenza A (H5N1) viruses in Vietnam (2006-2007). *Emerg. infect. Dis.*, **14**, 632-636.

21. Sims L.D., Ellis T.M., Liu K.K., Dyrting K., Wong H., Peiris M., Guan Y. & Shortridge K.F. (2003). – Avian influenza in Hong Kong 1997-2002. *Avian Dis.*, **47** (3 Suppl.), 832-838.

22. Sims L.D., Guan Y., Ellis T.M., Liu K.K., Dyrting K., Wong H., Kung N.Y., Shortridge K.F. & Peiris M. (2003). – An update on avian influenza in Hong Kong 2002. *Avian Dis.*, **47** (3 Suppl.), 1083-1086.

23. Smith G.J.D., Fan X.H., Wang J., Li K.S., Qin K., Zhang J.X., Vijaykrishna D., Cheung C.L. *et al.* (2006). – Emergence and predominance of an H5N1 influenza variant in China. *Proc. natl Acad. Sci. USA*, **103**, 16936-16941.

24. Smith G.J.D., Naipospos T.S.P., Nguyen T.D., de Jong M.D., Vijaykrishna D., Usman T.B., Hassan S.S., Nguyen T.V. *et al.* (2006). – Evolution and adaptation of H5N1 influenza virus in avian and human hosts in Indonesia and Vietnam. *Virology*, **350**, 258-268.

25. Subbarao K., Klimov A., Katz J., Regnery H., Lim W., Hall H., Perdue M., Swayne D. *et al.* (1998). – Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science*, **279**, 393-396.

26. Vijaykrishna D., Bahl J., Riley S., Duan L., Zhang J.X., Chen H., Peiris J.S.M., Smith G.J.D. & Guan Y. (2008). – Evolutionary dynamics and emergence of panzootic H5N1 influenza viruses. *PLoS Pathog.*, **4** (9), e1000161.

27. Wang J., Vijaykrishna D., Duan L., Bahl J., Zhang J.X., Webster R.G., Peiris J.S.M., Chen H. *et al.* (2008). – Identification of the progenitors of Indonesia and Vietnam avian influenza A (H5N1) viruses from southern China. *J. Virol.*, **82**, 3405-3414.

28. Webster R.G., Guan Y., Peiris M., Walker D., Krauss S., Zhou N.N., Govorkova E.A., Ellis T.M. *et al.* (2002). – Characterization of H5N1 influenza viruses that continue to circulate in geese in southeastern China. *J. Virol.*, **76**, 118-126.

29. World Health Organization Global Influenza Program Surveillance Network (2005). – Evolution of H5N1 avian influenza viruses in Asia. *Emerg. infect. Dis.*, **11**, 1515-1521.

30. World Health Organization (WHO)/World Organisation for Animal Health (OIE)/Food and Agriculture Organization (FAO) H5N1 Evolution Working Group (2008). – Towards a unified nomenclature system for the highly pathogenic avian influenza H5N1 viruses. *Emerg. infect. Dis.*, **14** (7). Online report. Available at: http://www.cdc.gov/EID/content/14/7/e1.htm (accessed on 23 January 2009).

31. Xu X., Subbarao K., Cox N.J. & Guo Y. (1999). – Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology*, **261**, 15-19.