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Review

Implications of global and regional patterns of highly pathogenic avian influenza virus H5N1 clades for risk management

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ABSTRACT

This paper analyses the publicly available data on the distribution and evolution of highly pathogenic avian influenza virus (HPAIV) H5N1 clades, whilst acknowledging the biases resulting from the non-random selection of isolates for gene sequencing. The data indicate molecular heterogeneity in the global distribution of HPAIV H5N1, in particular in different parts of East and Southeast Asia. Analysis of the temporal pattern of haemagglutinin clade data shows a progression from clade 0 (the 'dominant' clade between 1996 and 2002) to clade 1 (2003–2005) and then to clade 2.3.4 (2005 onwards). This process continuously produces variants, depending on the frequency of virus multiplication in the host population, which is influenced by geographical variation in poultry density, poultry production systems and also HPAI risk management measures such as vaccination. Increased multilateral collaboration needs to focus on developing enhanced disease surveillance and control targeted at evolutionary 'hotspots'.

Introduction

Since its emergence over a decade ago, the highly pathogenic avian influenza virus (HPAIV) strain type H5N1 has spread to 61 countries across three continents, been associated with more than 290 human fatalities,[1] and resulted in disease mortality and culling of several hundred million domestic poultry. Epidemiological studies conducted in a wide range of settings have provided important insights with respect to the complex interplay between wild bird and domestic poultry species within the various ecological systems where the virus has led to outbreaks (Sims et al., 2005; Martin et al., 2006; Alexander and Brown, 2009).

The epidemiological characteristics of the virus, including host range, survival in the environment, minimum infectious dose, pathogenicity and excretion rates, appear to support its large-scale endemicity within some ecosystems, such as the rice–paddy field production systems of Southeast Asia (Gilbert et al., 2008). There is a relatively small probability that such systems contain specific species reservoirs of infection, such as wild bird species. It is more likely that presence of disease at a larger geographical scale is the result of spatially and temporally dynamic pockets of infection, which result from illegal movement of infected poultry or their products, as well as, probably, the occasional exchange with infected wild birds (Gauthier-Clerc et al., 2007). National and multilateral agencies have responded in many ways to this zoonotic health and economic risk, and important lessons have already been and still need to be learned about disease control policy effectiveness.

HPAI is caused by an RNA virus with a relatively high rate of nucleotide substitution. The resultant accumulation of random mutations within the eight segments of its small genome combined with natural selection pressure influenced by host immunity responses results in variability of salient characteristics, including antigenicity, infectivity and virulence (Holmes, 2010). Since the putative introduction of the HPAIV H5N1 into Southeast Asia from China and its subsequent spread to South Asia, Europe and Africa, a large number of virus isolates has been genotyped. The genetic variability and relatedness of the virus isolates elucidates the paths of its spread and entrenchment in different ecological niches, particularly in Southeast and South Asia. Realising this, Bogner et al. (2006), Capua et al. (2006) and Capua and Alexander (2008) emphasized the need to make genetic data available to the global research community.

In this review article, we use published data to perform a qualitative analysis of the spatio-temporal distribution of HPAIV H5N1 clades as defined by nucleotide sequence variability of the haemagglutinin (HA) gene with the aim to identify patterns that can be used to develop more effective control and prevention strategies. The HA gene was used due it being the primary target of

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[1] See: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2010_04_09/en/index.html.

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the host immune response (Russell et al., 2008). The clade data were generated using the methodology defined by the WHO/OIE/FAO H5N1 Evolution Working Group (2008, 2009). The underlying classification algorithm was applied by Lu et al. (2007a,b) who make regularly updated data publicly available via the FluGenome website.[2] In the current analysis, the data were complemented by data published by Wan et al. (2008), Nguyen et al. (2008), Saito et al. (2008) and Gutiérrez et al. (2009).

Evolution and global spread of HPAIV H5N1

The evolution of influenza viruses is influenced by the interplay of high virus mutation rates, natural selection and host population characteristics, such as species susceptibility and population contact structure. This is either reflected in so-called antigenic drift or in exchange of HA or neuraminidase (NA) antigens between different influenza A virus sub-types co-infecting a particular host resulting in genetic re-assortment, also called antigenic shift (Treanor, 2004; Nelson and Holmes, 2007; McHardy and Adams, 2009).

Antigenic drift is common in influenza A and B viruses, and is primarily responsible for seasonal flu epidemics since it affects the HA and NA genes responsible for the main surface proteins to which host immunity and vaccines are directed. Genetic re-assortment can produce new influenza A viruses, such as in the case of the recent pandemic influenza A H1N1v outbreak. Both mechanisms, antigenic drift and genetic re-assortment, to varying degrees result in unpredictable changes to virus behaviour such as transmissibility, host range, virulence, treatment and vaccine efficacy. With the current HPAIV H5N1 epidemic, antigenic drift still appears to be the main mechanism, and there is scientific evidence that it has already resulted in changes in virus behaviour, such as effectiveness of treatment in humans (see, for example, Le et al., 2008; Boltz et al., 2010).

Phylogenetic and phylodynamic analyses based on comparisons between HPAIV H5N1 genotypes have been reported by several authors, including Smith et al. (2006), Duan et al. (2008), Gutiérrez et al. (2009), Guan et al. (2009), and Liang et al. (2010). Most recent analyses are based on the nucleotide sequence variability of the HA surface gene and apply a set of criteria for defining HA clade groups devised by a WHO/OIE/FAO H5N1 Evolution Working Group (2008, 2009). In brief, the phylogenetic analysis has led to the definition of lineages emerging from the earliest known progenitor isolate identified in the 1996 Guangdong outbreak (A/goose/Guangdong/96). Amongst these, a comparison of nucleotide distances at the HA locus within and between groupings of sample sequences using a phylogenetic tree resulted in the identification of at least 10 clades (Wan et al., 2008; Vijaykrishna et al., 2008). HA clade 0 includes those isolates which are genetically close to the progenitor virus, i.e. A/goose/Guangdong/96, which itself is believed to have emerged through introduction of low pathogenic avian influenza virus H5N1 from wild bird into domestic poultry populations in China (Guan et al., 2009). Each clade may encompass a number of subclades as well as sub-subclades.

Analysis of the temporal and spatial distribution of different clades can support the development of hypotheses about spread patterns within and between countries and regions (Smith et al., 2006; Ducatez et al., 2007; Salzberg et al., 2007; Wallace and Fitch, 2008; Buchy et al., 2009; Cattoli et al., 2009). The relative frequency of different clades within countries and regions can offer indications with respect to frequency of local transmission as distinct from introductions from other geographical areas.

It should be noted that the criteria for selection of sequenced isolates included in the database used for this analysis are not documented. It is also unlikely that the isolates are a representative sample of the spatial or temporal distribution of HPAIV H5N1 in the population at risk of any of the countries. This drawback needs to be kept in mind when interpreting the clade patterns discussed here. If a clade was reported frequently, it cannot be concluded with certainty, due to the absence of representative sampling, that it is common or 'dominant' at a particular time in a particular geographical area. In addition, there are various sources of selection bias, including some countries not submitting any gene sequences during selected time periods. For example, for 2009, at the time of writing, only gene sequences from Egypt were available in publicly accessible databases, which should not be interpreted as indicating that none of the other countries experienced any outbreaks.

Due to public health concerns, it is also more likely that virus isolates from human cases were sequenced than from animals. Despite these caveats, a detailed assessment of the space–time distribution allows some important inferences about regional dynamics of HPAIV H5N1 risk. In the current analysis, the main focus was placed on a comparative assessment of the relative occurrence of different clades within-country rather than of individual counts between countries, since the number of sequences varied substantially between countries. Due to the relatively small number of isolates per country and year, rare clade types were less likely to be detected and therefore any inferences are mainly about the common clade types. Holmes and Grenfell (2009) acknowledge the biases inherent to many molecular epidemiology studies of RNA viruses, particularly in relation to assessing spatial dynamics, but still find them useful for interpretation of epidemiological patterns.

As can be seen in Table 1, all 10 HPAIV H5N1 clades have been isolated in East Asia at one time or another. The sequences from East Asia are primarily from China and Hong Kong, with the exception of clade 2.2 which was also found in Japan and South Korea. The only clades that have not been found in East Asia (namely China, including Hong Kong) are 2.1.2 and 2.1.3, which appear to have evolved in Indonesia after their putative introduction from Hunan province of China in 2002–2003 (Wang et al., 2008). Some clades have so far only been isolated in China (e.g. clades 4, 6 and 9) whilst members of the clades associated with major epidemics (clade 1 in the Mekong region in 2004 and 2005, clade 2.1 in Indonesia as of 2004, clade 2.2 extending from Asia to Europe and Africa starting in 2005, and clade 2.3.4 affecting Vietnam, Lao PDR and Thailand since 2005) were all isolated in China before being found elsewhere.

Table 1 and Fig. 1 reveal the global dispersion of HPAIV H5N1. Despite having infected many countries the number of clades, and therefore the genetic variability of the virus, causing outbreaks outside East and Southeast Asia, is much smaller than within that geographic region. Indeed, nearly all gene-sequenced isolates obtained west of Myanmar at the time of writing are drawn from a single clade group (2.2). This suggests that the high transmission frequencies in some parts of East and Southeast Asia resulting from high population densities of terrestrial and aquatic poultry combined with regional husbandry and trading practices have led to increased chances of antigenic drift. Indeed, the three southern provinces of China, namely, Yunnan, Guangxi and Guangdong, alone have a combined standing poultry population of more than 600 million domestic poultry, similar in size to that of India as a whole. The development of genetic diversity of HPAIV H5N1 in China has been analysed by Chen et al. (2006), Duan et al. (2008) and Guan et al. (2009).

Based on the above, the following three hypotheses about HPAIV H5N1 evolution and epidemiology can be formulated:

1. The risk for emergence of HPAI H5N1 re-assortant viruses may be particularly high in regions with higher clade diversity, perhaps justifying more intensive use of multilateral resources for disease surveillance and control in these areas.

[2] See: http://www.flugenome.org.

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Table 1
Temporal pattern of occurrence of HPAIV H5N1 clades in poultry and humans by geographical region. Source: Data from http://H5N1.flugenome.org (accessed 14th April 2010) plus additional data on Vietnam, Cambodia and Myanmar from Gutiérrez et al. (2009) and Saito et al. (2008).

Region	Clade	1996–1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
East Asia	0	51	17	18	17	5	4	1		1	1	
	1				17	16						
	2.1.1				1	1						
	2.2							20	9	4		
	2.3.1					1	3	13				
	2.3.2					1	11	43	3			
	2.3.3						1	6				
	2.3.4							47	105	21		
	2.4				2	14	9	2				
	2.5					8	8		3			
	3		3	41		1						
	4				7	1		1	7			
	5		1	2	2	5	16					
	6				1		8					
	7					2	4	9	3			
	8				7	1						
	9			1	2	7	16	9	1			
	Outliers			2	8	8	4	1				
Southeast Asia	0							3	5			
	1					13	283	157	18	26	14	
	2.1.1					11	16	7	1			
	2.1.2						2	22	18			
	2.1.3						6	34	110	15		
	2.3.2							14	1			
	2.3.4							10	3	27		
	3			2								
	5				1	1						
	7								1			
	8							1				
	Outliers						2					
South Asia	1									1		
	2.2								5	14	29	
Europe	1						4					
	2.2							52	158	37		
Central Asia	2.2							7	8			
West Asia	2.2							1	20	4	2	
North Africa	2.2							1	50	87	53	44
West Africa	2.2								112	67	2	
East Africa	2.2								1			
Total		51	21	66	65	96	397	461	641	302	101	44

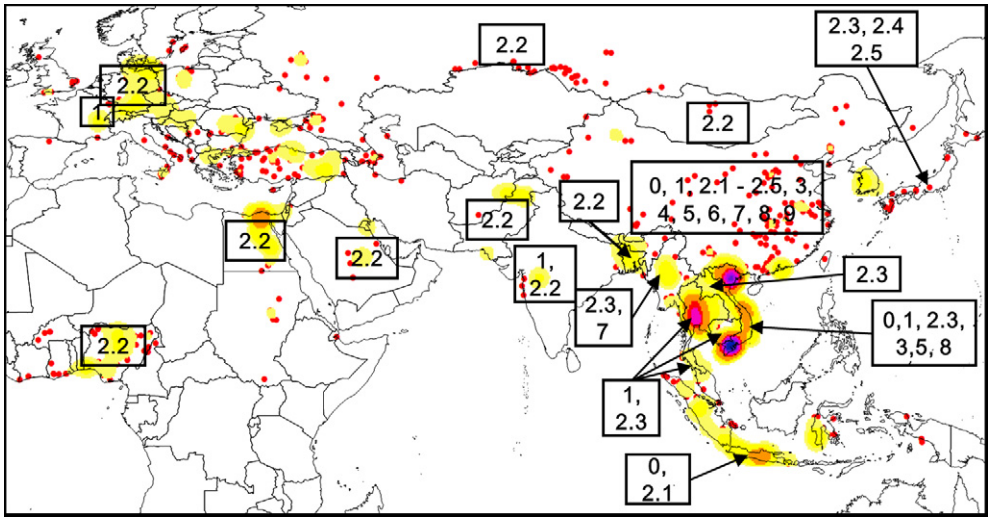

Fig. 1. Global dispersion of HPAIV H5N1 clades together with cumulative spatial distribution of highly pathogenic avian influenza virus (HPAIV) H5N1 outbreaks in poultry and wild birds between 2003 and 2009 (density of outbreaks shaded between yellow (low but >0) and dark blue (high) and isolated outbreaks shown as red dots).

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Table 2
Cost of human HPAI H5N1 epidemics in selected countries assuming three hypothetical mortality scenarios.

Country	Human population (millions)	HPAI H5N1-related deaths (million)[a]			GDP per capita (US$)	SVL[b] (US$ million)	Cost of human epidemic (US$ billion)[a]		
		1%	0.5%	0.1%			1%	0.5%	0.1%
USA	304	3.04	1.52	0.30	47,000	6.90	20988	10,494	2099
China	1339	13.39	6.70	1.34	6000	0.88	11798	5899	1180
Japan	127	1.27	0.64	0.13	34200	5.02	6392	3196	639
India	1166	11.66	5.83	1.17	2800	0.41	4792	2396	479
Germany	82	0.82	0.41	0.08	34800	5.11	4210	2105	421
UK	61	0.61	0.30	0.06	36,600	5.37	3276	1638	328
Russia	140	1.40	0.70	0.14	15,800	2.32	3248	1624	325
France	64	0.64	0.32	0.06	32,700	4.80	3088	1544	309
Brazil	199	1.99	0.99	0.20	10,100	1.48	2951	1475	295
Mexico	111	1.11	0.56	0.11	14,200	2.08	2319	1159	232
Thailand	66	0.66	0.33	0.07	8500	1.25	819	410	82
Pakistan	176	1.76	0.88	0.18	2600	0.38	673	336	67
Egypt	83	0.83	0.42	0.08	5400	0.79	659	329	66
Vietnam	87	0.87	0.44	0.09	2800	0.41	358	179	36
Israel	7	0.07	0.04	0.01	28,200	4.14	305	153	31
Total	4014	40.14	20.07	4.01			65,875	32,937	6587

[a] Three scenarios, high HPAI H5N1-specific mortality: 1% of human population dies, e.g. 2% affected, 50% case fatality; medium HPAI H5N1-specific mortality: 0.5% of human population dies; low HPAI H5N1-specific mortality: 0.1% of human population dies.
[b] SVL, 'statistical' value of life.

2. The occurrence of multiple clades in a given location that are also found elsewhere may indicate that transboundary linkages (probably through poultry trade and/or wild bird movements) are important determinants of local and regional HPAI H5N1 disease dynamics.
3. Medium to long-term local re-occurrence of outbreaks associated with a single clade (or subclade) is probably a sign of endemicity.

Cost of emergence of HPAIV H5N1 transmissibility between humans

Fortunately, HPAIV H5N1 currently does not transmit easily from poultry to humans and there has been no proven human-to-human transmission. Consequently, the number of reported human cases has been very small considering the large number of people exposed to poultry in the affected regions, through keeping, trading or purchasing them in live bird markets. However, the possibility of acquisition of higher transmissibility to and between humans by the virus still remains, either through antigenic drift or, more likely, through re-assortment with other influenza A viruses circulating in humans and/or domestic animals, such as pigs, which are also abundant in East and Southeast Asia. If such an event were combined with high human case fatality (around 50%), it would result in extremely high human epidemic costs even in low incidence scenarios.

Orders of magnitude of the cost of human HPAI H5N1 epidemics can be derived using the 'statistical value of life approach' (Sproul et al., 2009), which assigns a monetary value to the life (or death) of an 'average citizen' in relation to a country's economic situation. Based on this (admittedly simplistic) approach, the cost of a 'low' incidence human HPAIV H5N1 epidemic (0.2% of the human population become infected, half of which die – by comparison, seasonal flu tends to infect about 10% of a population) in China PDR would amount to US$1180[3] billion from mortality alone. For Vietnam, the same scenario would result in a human epidemic costing US$36 billion while for the USA, the cost of such an epidemic would be higher than US$2000 billion (Table 2).

[3] $1 = approx. £0.64, €0.76 at 19th December 2010.

Evolution and dispersion of HPAIV H5N1 in the Greater Mekong Sub-Region

Table 3 tabulates clade identification in China and the other Mekong countries between 1996 and 2008. It shows the link between the occurrence of clades in China and its neighbour Vietnam, but also that the Mekong countries are likely to have been a reservoir for clade 1 between 2003 and 2008. For the geographical region it reveals a progression from clade 0 (the 'dominant' clade between 1996 and 2002) to clade 1 (2003–2008) and then to clade 2.3.4 (2005 onwards). The number of clades in this region and their transition both suggest high frequency transmission of virus, generating successful new genetic variants through a process involving high mutation rates typical for RNA viruses and positive selection (Nelson and Holmes, 2007; Holmes, 2010). This phenomenon has been recognised for human influenza A virus where the presence of a continuous infection reservoir in East and Southeast Asia has been hypothesized (Rambaut et al., 2008; Russell et al., 2008; Holmes, 2009). Nevertheless, it has recently been postulated that for human influenza A virus evolution the high migration rates amongst the human population combined with use of antiviral treatment have probably resulted in other regions, such as the USA, also becoming important (Bedford et al., 2010).

Nevertheless, in the case of HPAIV H5N1, neither the limited movement of domestic poultry nor the migration of wild birds is likely to have a dispersal capacity for the virus that is anywhere near to human movement, as has been eminently demonstrated by the rapid spread of influenza A H1N1v amongst humans in 2009 across the globe (Lemey et al., 2009). The presence of a continuous reservoir of HPAIV H5N1 in East and Southeast Asia poses a serious on-going challenge for disease risk management and, from a pandemic perspective, should be a focal point for intensive policy intervention. Moreover, in light of the public good nature of contagious disease prevention, it suggests an essential area for multilateral cooperation.

Fig. 2 depicts the spatial distribution of HPAI outbreaks in the Greater Mekong Sub-Region during the initial epidemic waves (2004–2005) and the subsequent phase of reduced outbreak incidence (2006–2009). Two major HPAI 'hotspots' remain in Vietnam, one in the north (Red river delta) and one in the south (Mekong river delta). These are characterised by highly productive smallholder

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Table 3
Temporal pattern of HPAIV H5N1 clades in animals and humans in China (incl. Hong Kong) and the Mekong countries. Source: Data from http://H5N1.flugenome.org (accessed 13th April 2010) plus data from Vietnam, Cambodia and Myanmar from Gutiérrez et al. (2009), Nguyen et al. (2008) and Saito et al. (2008).

Country	Clade	1996–1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
China	0	51	17	18	17	5	4	1		1	1
	1				17	16					
	2.1.1				1	1					
	2.2							20	5	3	
	2.3.1					1	3	13			
	2.3.2					1	11	43	3		
	2.3.3						1	6			
	2.3.4							47	105	19	
	2.4				2	14	9	2			
	2.5					5	2		3		
	3		3	41		1					
	4				7	1		1	7		
	5		1	2	2	4	16				
	6				1		8				
	7					2	4	9	2		
	8				7	1					
	9			1	2	7	16	9	1		
	Outliers			2	8	8	4	1			
Cambodia	1							4	4	1	
Laos	2.3.4								1		
Myanmar	2.3.4									3	
	7								1		
Vietnam	0							3			
	1					13	102	81	4	20	
	2.3.2							14	1		
	2.3.4							10		21	
	3			2							
	5				1	1					
	8							1			
Thailand	1						179	72	10	5	14
	2.3.4								2	2	
Total		51	21	66	65	81	359	337	149	75	15

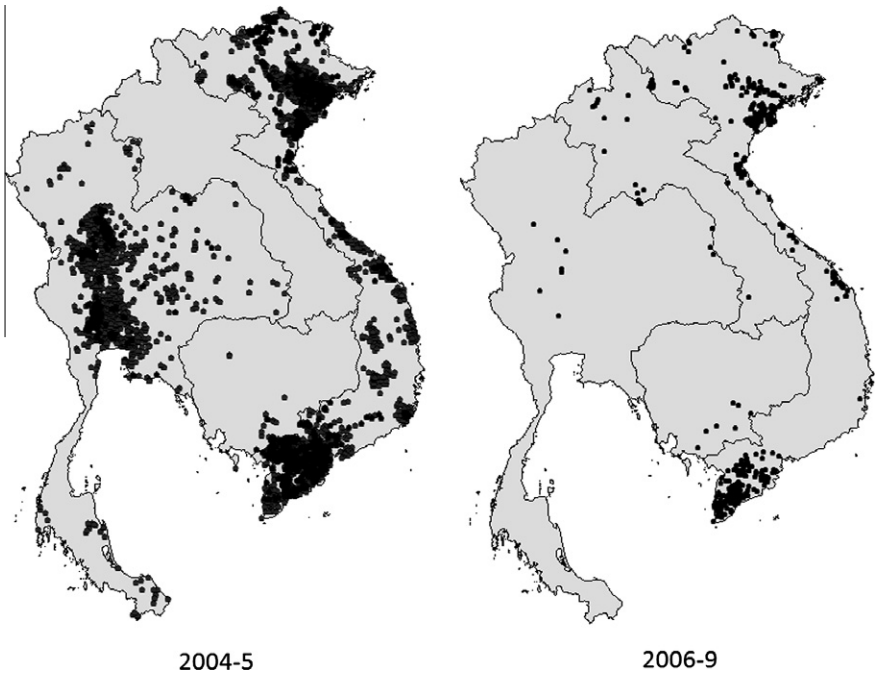

Fig. 2. Spatial distribution of highly pathogenic avian influenza virus (HPAIV) H5N1 outbreaks (black dots) in poultry and wild birds in the Greater Mekong Sub-Region for the periods 2004–2005 and 2006–2009 (based on official government reports to OIE).

rice–paddy production systems combined with terrestrial and aquatic poultry (Pfeiffer et al., 2007).

Differences in the relative importance of clades types within these hot spots are illustrated in Table 4, although it should be noted that locational information was only available for a small subset of sequenced isolates. Given that caveat, Table 4 should be examined considering the occurrence of different clades in neighbouring countries. Clade 1 was first reported from China in

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Table 4
Subset of HPAIV H5N1 clades with province location information from Vietnam from animals and humans by geographical region. Source: Combined data from Wan et al. (2008), Nguyen et al. (2008) and http://H5N1.flugenome.org (accessed 14th April 2010).

Region	Clade	2003	2004	2005	2006	2007
North	1	13	12	12		
	2.3.2				1	
	2.3.4					21
	5	2				
Central	1		7			
	2.3.4					1
South	1		31	24	5	19
	2.3.4					1

2002. It has been the 'dominant' clade in Thailand and Vietnam since 2003, but has not been reported from China since that year. Table 4 reveals that 'dominant' clade transition in northern Vietnam mirrors that of its northern neighbour (from clade 1 to clade 2.3.4), while in southern Vietnam clade 1, the agent of the initial epidemic waves, persists (which mirrors the situation of Thailand and Cambodia). This comparison evokes a number of policy implications, the most prominent of which are that there are important transboundary linkages between China and northern Vietnam (with Lao PDR having an intermediary role), and that HPAIV H5N1 is endemic in southern Vietnam's Mekong river delta, and thereby is likely to have become a recurrent source of infection for Cambodia, in particular.

Given that the role of wild birds in disease spread is likely to be of minor importance within the Greater Mekong Sub-Region and that governments have banned trade of poultry or poultry products across their borders, transboundary spread of HPAIV H5N1 must result primarily from informal movements of poultry and poultry products across country borders.

These findings reinforce a perception that disease risk management in the Greater Mekong Sub-Region could be more effective with a multilateral approach that limits re-infection risk and, where possible, coordinates activities to an extent that facilitates containment and possibly progressive eradication.

Defining HPAI risk categories

Our assessment of the combined effects of viral evolution and spatial dispersion presents a heterogeneous global landscape of HPAI H5N1 disease risk, and policies toward both livestock sector defence and pandemic aversion must adapt to this reality. To facilitate more effective policy design, we propose a classification scheme for functional differences in national and within-country HPAI H5N1 disease situations and 'risks' which is shown in Table 5. According to this classification, policy orientation toward poultry sector protection can be stratified into preventive (Categories 0 and 1) and reactive (Categories 2–4) regimes. The former would emphasize managing 'inbound' risk with a focus on transboundary risk management, surveillance for early disease detection and limited commitments to contingent response. In the second group, more extensive commitments are required to respond to and contain current outbreaks, possibly including multilateral coordination to limit transboundary transmission.

For the two highest categories (3 and 4), intensive measures need to be targeted toward containment and eradication of local reservoirs, combined with transboundary risk management. In this context, rigorous assessments are required to determine if and how to tackle endemicity and conditions that sustain virus reservoirs. Without these investments, risks of both re-infection and development of HPAIV strains capable of human-to-human spread will continue to present very high present-value social costs. Again, multilateral commitments may be necessary to more effectively avert or limit these risks. Countries in these two categories currently include, for example, Vietnam, Thailand, China and Indonesia.

Categories 3 and 4 countries may also have to resort to preventive large-scale vaccination programmes, because the contact network within the poultry production and trade system is so intense in space and time that virus transmission cannot be prevented through other control measures. But the use of this control option needs to be evaluated and planned carefully, particularly if it has to be applied over very large geographical areas and diverse poultry production systems (Savill et al., 2006; Chen, 2009; Hinrichs et al., 2010; Walker et al., 2010). In such conditions, inadequate and/or spatially varying vaccination coverage is likely to result in prolonged virus endemicity at low incidence levels, which will require long-term continuation of the large-scale vaccination campaign, typically associated with further decreasing coverage due to decreasing support from stakeholders in the absence of 'visible' outbreaks. At the same time, this virus endemicity in the presence of poorly cross-protective vaccination may increase selection pressure towards virus variants (or 'escape mutants'), against which the vaccine does not protect (Smith et al., 2006; Restif and Grenfell, 2007; Iwami et al., 2009; Domenech et al., 2009).

Category 4 countries are a special regional and global priority for co-ordinated disease risk management. Because they represent the most dynamic evolutionary conditions for the virus, it is of global interest to support these countries in their containment and eradication efforts. Conversely, Category 4 countries have special responsibilities to make full use of multilateral resources, sustain constructive and open policy dialogue, and adhere to international standards for timely information sharing.

In the presence of significant variation in poultry density, trade patterns and production systems within a country, it may be necessary to sub-divide a country into regions with different risk

Table 5
Classification scheme for functional differences in national and within-country highly pathogenic avian influenza virus (HPAIV) H5N1 disease situations and 'risks'.

Risk category	Definition[a]	Economic/public health impact	Direction of spread	Re-assortment risk ranking
0	No past or current HPAIV H5N1 outbreak occurrence (in last 5 years)	None	Inbound	Negligible
1	Past outbreaks, but no current outbreaks or discernable reservoir risk (in last 5 years)	Negligible	Inbound	Negligible
2	Current, recurrent, or proximate (<6 months) outbreaks	Poultry population losses and/or vaccination costs	Inbound and outbound	Very low
3	Apparently endemic: persistent localized recurrence of known clades with discernable reservoir potential	Poultry population losses and/or vaccination costs, public health costs	Outbound	Medium
4	Endemic and significant evolutionary pressures: appearance of novel clades	Poultry population losses and/or vaccination costs, public health costs	Outbound	Higher than all other categories

[a] Assumes adequate sensitivity of surveillance.

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categories, and develop tailored prevention and control policies for each.

Conclusions

While the design of effective control and prevention strategies for infectious diseases needs to be informed by epidemiological analysis and modelling research at animal level, additional essential insight into transmission dynamics can be provided by combining epidemiological with phylogenetic data. In the case of RNA viruses with high mutation rates, such as HPAIV H5N1, important effects may be inferred at large and small spatial and temporal scales, as discussed in this review. In addition, if vaccination is used as part of a control and prevention strategy for such virus diseases, phylogenetic analysis also becomes important for surveillance, since the vaccine may only be efficacious for a relatively narrow range of genetic variants which in turn results in selection pressure for variants against which the vaccine does not cross-protect.

It is acknowledged that the data analysed here is likely to be affected by significant selection bias, but the evidence is sufficiently robust to inform HPAI H5N1 risk management in the Greater Mekong Sub-Region. In particular, it is apparent that the great diversity of HPAIV clades combined with the large and rapidly growing poultry populations in the region significantly increases the importance of regional policy dialogue and coordination. Without this, national risk management approaches, particularly in the northern provinces of Vietnam and southern China, will be of limited effectiveness, result in significant economic costs for national governments and other stakeholders and domestic and international public resources cannot be effectively utilized.

Without regional coordination, risk of re-infection with HPAIV H5N1 and the advent of new virus strains will impose costs on individual countries that will not allow lasting disease abatement. Therefore, individual governments and multilateral agencies should make determined efforts to support regional management of transboundary risk and coordination of national efforts to limit HPAIV H5N1 incidence.

Ideally, risk management policy adaptation should be evidence-based, but current global and regional HPAIV H5N1 policies do not appear to take sufficient account of geographic variability in molecular virus characteristics. Most of the currently observed clades have been identified only in China, Thailand and Vietnam, with the majority confined to China. All three countries have had to commit very substantial human, financial and science resources towards dealing with this challenge for several years now, and been able to reduce, but not eliminate the risk at a regional level. The international community needs to recognise that this situation continues to pose a serious on-going challenge for HPAIV H5N1 risk management and, from a pandemic risk perspective, should be a focal point for intensive dialogue, effective knowledge exchange and coordinated intervention. Moreover, in light of the public good nature of contagious disease prevention, it suggests an essential area for multilateral cooperation.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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