



# focus on

VOL 7 | JAN 2014

## Contributors:

Caryl Lockhart, Ken Inui, Jim McGrane,  
Mohinder Oberoi, Gwenaelle Dauphin,  
Vincent Martin, Sherrilyn Wainwright,  
Juan Lubroth, Subhash Morzaria,  
Filip Claes, Julio Pinto

Received: June/July 2013;  
Accepted: November 2013; Published: January 2014

## Update on the continuous spread and expansion of H5N1 highly pathogenic avian influenza

### Clade 2.3.2.1 in Asia (2010–2012)

1

Introduction

2

New H5N1 clade 2.3.2.1 in Indonesia

4

Highly pathogenic avian influenza and virus clades responsible for outbreaks in Asia during 2010–2012

5

Geographic distribution and expansion of clade 2.3.2.1

9

Risk assessment for the spread of clade 2.3.2.1 in Asia

9

Transmission pathways for clade 2.3.2.1 in Asia

9

Risk management

9

Areas and bird populations at risk

10

Risk management options

12

References

### Introduction

**H**5N1 highly pathogenic avian influenza (HPAI) virus, initially detected in 1996 in China, spread to more than 60 countries or territories on three continents within a ten-year period and has become endemic in poultry in several countries and regions (including Ganges–Brahmaputra Delta, Indonesia, Viet Nam, China and Egypt). The virus infects wild birds and domestic poultry and causes sporadic transmissions to humans raising concerns of a potential pandemic (Guan *et al.*, 2004; Peiris *et al.*, 2007). The recent confirmation of human cases of low pathogenic avian influenza A (H7N9) and bird positive findings across multiple provinces in China since April 2013 in live bird markets highlights the threat posed by existing and newly emerging avian influenza viruses irrespective of their virulence. The economic impact of disease caused by avian influenza viruses is related to losses incurred as a result of high mortality in poultry, to costs associated with control measures

including poultry movement restrictions, to disruption of trade and threats to food security of resource poor countries (Horimoto and Kawaoka, 2001; Campitelli *et al.*, 2002; McLeod *et al.*, 2004).

The H5N1 HPAI virus has changed and evolved in poultry into genetically distinct virus clades (0–9) and subclades (WHO/OIE/FAO H5N1 Evolution Working Group, 2012), particularly in Asia. Of the ten H5N1 HPAI virus 1<sup>st</sup> order clades<sup>1</sup> identified globally during 1996–2012, all have been found in China, which is considered to be the source of all H5N1 HPAI viruses identified (Sims *et al.*, 2005; Zhao *et al.*, 2008; Duan

<sup>1</sup> The standard clade nomenclature system defines clades based upon the evolution of H5 HA and are defined as 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> order clades. They are assigned a number which links them to their original clade using a hierarchical decimal numbering system. For example, within the distinct 1<sup>st</sup> order clade 2, an example of a 2<sup>nd</sup> order clade would be 2.3, 3<sup>rd</sup> order clades are designated as clades 2.3.1 and 2.3.2. A 4<sup>th</sup> order clade would be designated 2.3.1.1 and 2.3.2.1.

*et al.*, 2008). Once these new virus clades emerge, they either become established and circulate endemically within localized areas, as is the case with clade 2.1 in Indonesia, or they spread across national or international borders as was seen with clade 2.2. This clade spread from Qinghai Lake, China to the Russian Federation (Siberia) and then to a number of countries in Asia, the Middle East, Europe and Africa, between 2005 and 2006 (Webster *et al.*, 2006). Many of these newly evolved viruses have been responsible for large poultry outbreaks, particularly when introduced to new areas or to those areas where vaccination coverage was inadequate.

Of the various H5N1 HPAI virus clades circulating in Asia since 2004, clade 2.3.2 (and its 4<sup>th</sup> order variant 2.3.2.1) seems to have spread more widely geographically and has been the predominant clade isolated from H5N1 HPAI outbreaks in domestic poultry and wild birds in 14 countries across East, South and Southeast Asia during the last two years (2010–2012). Clade 2.3.2.1 viruses isolated in Asia during this period belong to three genetic clusters: A/barn swallow/Hong Kong/D10-1161/2010-like (BS), A/Hubei/1/2010-like (HB) and A/Hong Kong/6841/2010-like (HK) (WHO/OIE/FAO H5N1 Evolution Working Group, 2012). In countries where the virus clade 2.3.2.1 has recently been introduced, it has either replaced or co-circulated with existing endemic virus clades (e.g. with 2.3.4) creating some challenges in H5N1 HPAI disease control efforts, nationally and regionally.

The confirmation, for the first time, of clade 2.3.2.1 in ducks in Indonesia in November 2012, has serious implications for the further spread of the clade within Indonesia and throughout the region as a whole. Since 2004, Indonesia is endemically infected with clade 2.1 which is widespread throughout the country. An additional clade in this country means that available vaccines may not provide protection, resulting in increased opportunities for new viruses to appear (reassortants) and to become endemic. For the Asia region, that countries (whether endemic or not) remain at risk of introduction since clade 2.3.2.1 viruses continue to circulate and new strains continue to emerge from regional hotspots.

Additionally, the co-circulation of various subclades of the H5N1 virus in the region where poultry and human populations are dense may increase the risk of evolution of pandemic H5N1 strains. Given the presence of naive poultry populations in Indonesia and surrounding countries, the virus is expected to spread unless national veterinary authorities are able to implement biosecurity measures effectively. The fact that clade 2.3.2.1 continues to spread into new areas also suggests a potential risk of spread to regions outside of Asia. Although the risk is low for the spread of clade 2.3.2.1 from Indonesia to regions outside of Asia, the possibility of spread from endemic areas such as India and Bangladesh cannot be ignored. Countries at risk therefore need to maintain vigilance, improve surveillance for early detection and implement control measures in association with locally adapted vaccines. ●

### New H5N1 clade 2.3.2.1 in Indonesia

H5N1 HPAI has been endemic in Indonesia since 2004 and all outbreaks prior to this new introduction of H5N1 clade 2.3.2.1 are attributed to virus clade 2.1. Outbreaks due to clade 2.1 have decreased in most provinces across Indonesia since 2009.

H5N1 HPAI due to virus clade 2.3.2.1 was officially confirmed for the first time in Indonesia in November 2012, mainly in ducks on the island of Java, though there were media reports of duck mortalities possibly caused by clade 2.3.2.1 as early as August 2012. Sequence analysis of the HA gene of representative isolates from these outbreaks involving duck mortalities placed them within clade 2.3.2.1, clustering close to A/Hong Kong/6841/2010-like viruses, currently circulating in Viet Nam, China and Hong Kong SAR. Duck mortalities, used as an indicator of H5N1 clade 2.3.2.1 outbreaks after initial laboratory confirmation, were subsequently reported on four islands of Indonesia (Figure 1).

Clinical signs and mortality observed in Indonesia so far seem to mirror findings in other countries infected with virus clade 2.3.2.1. Mortality rates observed on the island of Java ranged between 10 percent and 90 percent. Ducks appeared to be the species most affected, showing mortality of 25

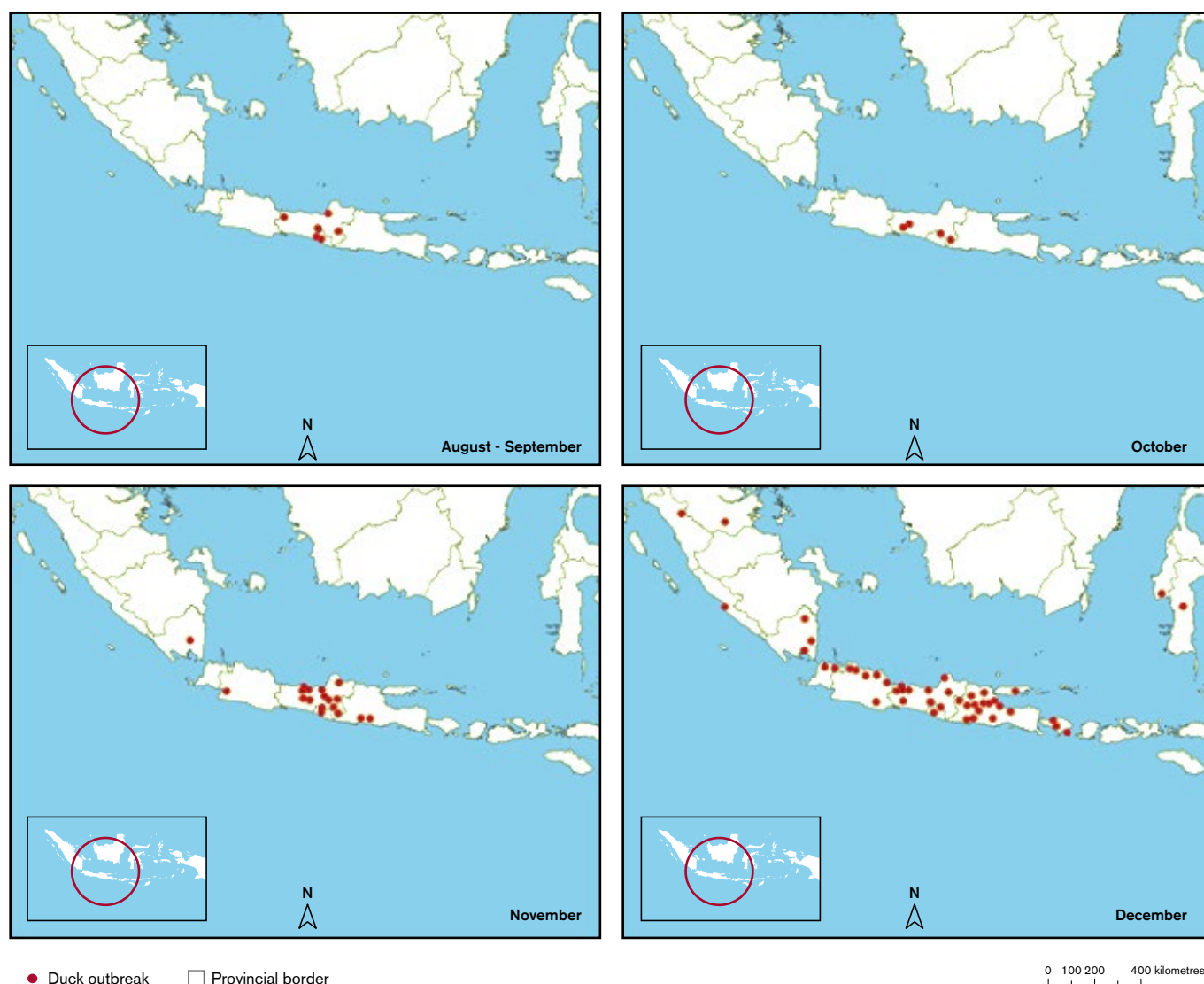
to 50 percent with higher rates observed in juveniles. Affected ducks did not die immediately, but showed nervous signs, such as torticollis two days prior to succumbing to infection. The number of dead ducks observed per outbreak was highly variable (range 5–70 000) and production types affected included backyard flocks (19 percent), commercial flocks (33 percent) and unknown production types (48 percent). Though there were unconfirmed reports of egg drop in layers and a small number of reports of mortality in backyard chickens in association with duck mortalities, no significant increases in the chicken outbreaks were reported. This result may be due to the novelty of lethal H5N1 clade 2.3.2.1 outbreaks in ducks and an increased focus on investigating duck outbreaks by the veterinary officers. Currently, it is not possible to differentiate between outbreaks caused by clade 2.1 and 2.3.2.1 since most H5N1 HPAI outbreaks in Indonesia are declared by case definition based on clinical signs and a positive rapid influenza A antigen test. Only a small proportion of samples are subjected to laboratory testing by PCR and sequence analysis. Because reports of duck mortalities due to H5N1 clade 2.1 prior to the new clade introduction were rare, observation of duck mortality is used to define an outbreak of HPAI H5N1 due to the clade 2.3.2.1. Thus, outbreaks in ducks are used as a proxy for the spread of clade 2.3.2.1 in Indonesia, which means that other HPAI H5N1 clades or non-HPAI causes cannot be ruled out.

Figure 2 shows the number of reported duck outbreaks (reports of duck mortalities) between August 2012 and January 2013. The initial spread through the end of November 2012 was relatively slow, with a sudden increase at the beginning of December, coinciding with a large meeting of the Government about the new outbreaks. Figure 1 shows the location of duck outbreaks defined on the basis of reported mortalities in ducks between August and the end of December 2012. Initially, the reports of duck outbreaks were limited to Java, but the outbreaks spread rapidly between the islands and, in December, four islands reported outbreaks in ducks (Java, Sumatra, Sulawesi and Bali).

The source of the introduction of the new virus clade to Indonesia is currently

Figure 1

Spatial distribution of duck mortalities (assumed to be outbreaks of H5N1 HPAI) in Indonesia between August and December 2012)



unknown. The virus had been introduced to three islands outside Java by the time the Government started its efforts to contain the outbreaks in Java. Of the main Indonesia islands, only Kalimantan, with its large duck population, has, as of March 2013, not yet reported outbreaks of the new clade. It is expected that with the start of the influenza season, the number of outbreaks will increase both in areas where the new clade already has been introduced, as well as in new areas.

This new incursion of virus clade 2.3.2 in Indonesia presents considerable challenges for the Indonesian Government in terms of outbreak response and containment, prevention and control. Although the Local Disease Control Centre (LDCC) and Participatory Disease Surveillance and Response Programme (PDSR) have improved coordination between central and local governments for local outbreak response activities, the decentralized government structure still complicates disease control

and the administration of funds for culling and compensation. Surveillance is the mandate of the Directorate General of Livestock and Animal Health Services (DGLAHS) Disease Investigation Centres (DIC) and in some cases this overlaps with surveillance activities carried out by district governments through PDSR. Intra-island (interprovince) animal movement control is difficult to implement due to the sheer volume of trade, and inter-island movement control falls under the responsibility of the National Animal Quar-



antine Service – a separate agency within the Ministry of Agriculture (MOA) – which has had difficulty in rapidly implementing animal movement restrictions in the past. Whereas biosecurity and vaccination have been reasonably effective in controlling HPAI in sectors 1 and 2<sup>2</sup> insufficient information is available from these sectors. Biosecurity in sector 3 and in ducks is very low. While many layer farmers in sector 3 vaccinate against H5N1 HPAI, broiler and duck farmers do not vaccinate their flocks.

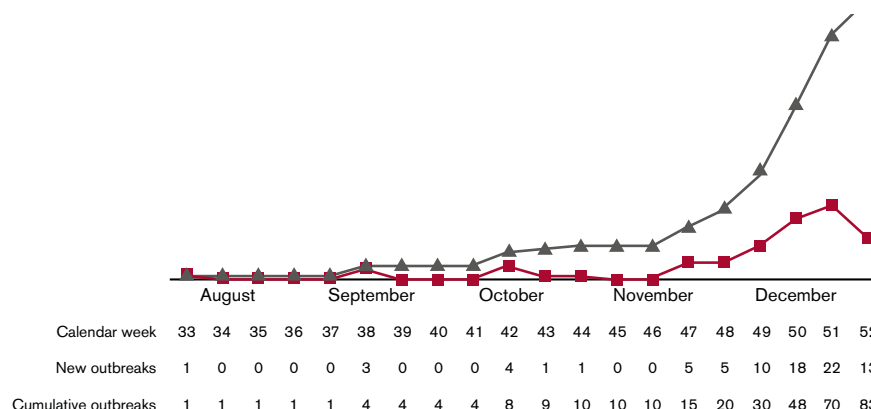
In Indonesia, the Food and Agriculture Organization of the United Nations (FAO) has provided recommendations for containment and control of H5N1 HPAI clade 2.3.2.1 to the Department of Agriculture (DAH) and has shared information about the distribution of clade 2.3.2.1 in Asia. FAO further provided advice on laboratory diagnosis and is facilitating the process to acquire new reagents from AAHL to detect the new clade. Information on the new virus clade is being provided to the local government staff of both PDSR and the Commercial Poultry Veterinary Programme (PVUK) through fact sheets and continuing education in order to strengthen surveillance and control in the field. ●

### Highly pathogenic avian influenza and virus clades responsible for outbreaks in Asia during 2010–2012

H<sup>5</sup>N1 HPAI has been prevalent in Asia since 2004, with reported outbreak numbers and affected countries showing a decreasing trend during the 2010–2012 period. Between 2010 and 2012, a total of 16 countries reported H5N1 HPAI outbreaks in Asia caused by six virus clades. See Figure 3 for clades causing outbreaks during 2010–2012 in Asia. H5N1 HPAI in Asia follows a seasonal pattern with most outbreaks occurring from November to March each year; possibly during the times when poultry movement and trade is at its highest or when wild bird migration is occurring (see Figure 4). The viruses responsible for these outbreaks emerged from the

Figure 2

Number of H5N1 HPAI outbreaks (mortalities) reported in ducks between August 2012 and January 2013



Source: FAO Indonesia

Figure 3

Distribution of H5N1 HPAI clades reported in East, Southeast and South Asian countries during 2010–2012, showing the possible source of the virus clade 2.3.2.1 newly detected in Indonesia during this period in Southeast Asia (Epizone<sup>a</sup> 7)



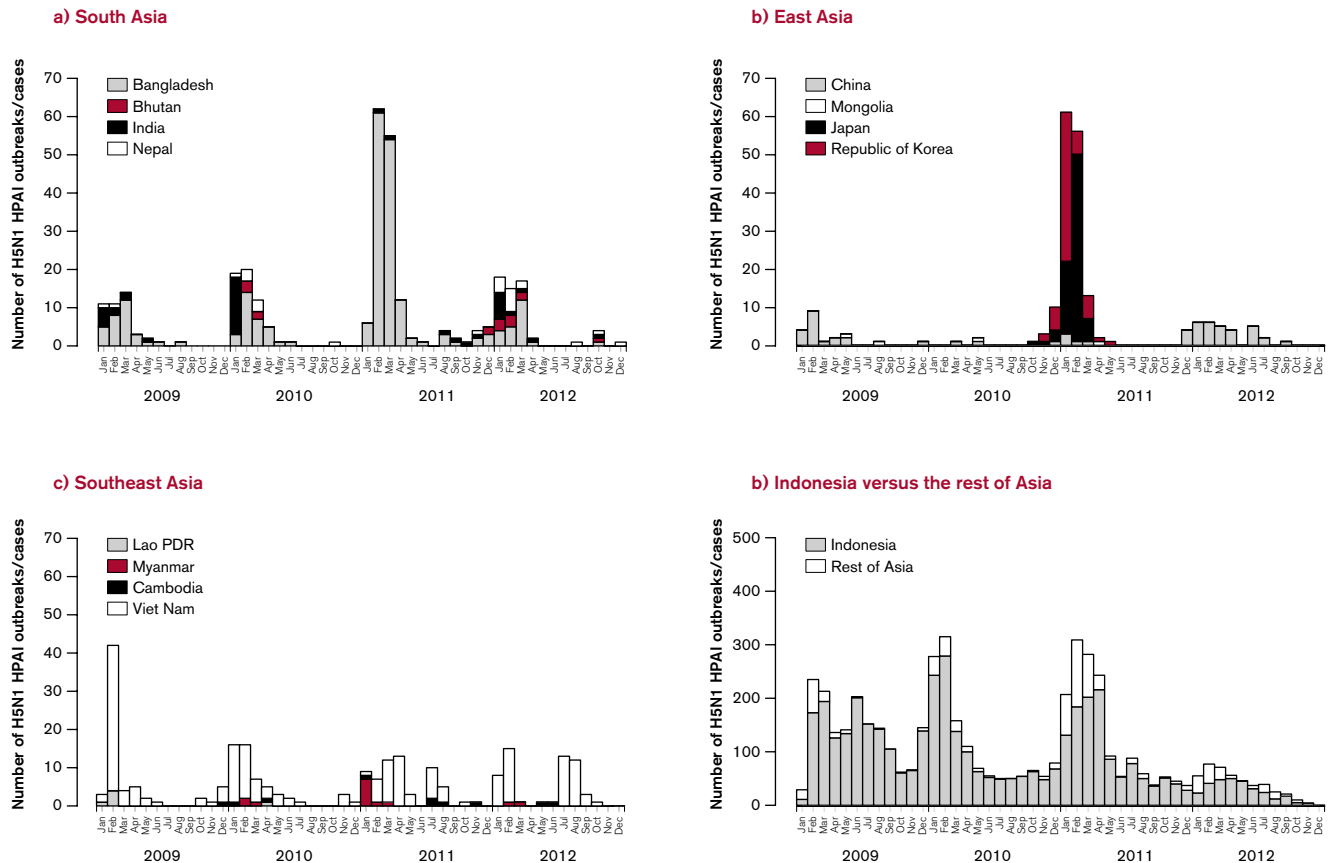
<sup>a</sup> Epizones are defined as geographical areas where closely related viruses were shared, and frequent virus incursion/exchange is expected. Epizones: 1: Japan, Republic of Korea, north China and the Russian Federation (Siberia); 2: China; 3: south China, China (Hong Kong), Viet Nam (north) and the Lao People's Democratic Republic; 4: Cambodia and Viet Nam (south); 5: Indonesia; 6: Myanmar and south China; 7: Myanmar, Bangladesh, India, Nepal and Bhutan; 8: Nepal, Mongolia, west China and the Russian Federation (Siberia); 9: Mongolia and the Russian Federation (Siberia).

Source: Inui, 2012

<sup>2</sup> <http://www.fao.org/docs/eims/upload/214190/ProductionSystemsCharacteristics.pdf>

Figure 4

## Bar graphs of monthly H5N1 HPAI outbreaks in poultry and wild birds 2005–2012



A/goose/Guangdong/96 lineage and have been classified into ten 1<sup>st</sup> order clades (0–9) (WHO/OIE/FAO H5N1 Evolution Working Group, 2012). Several 1<sup>st</sup> order clades (0, 3, 4, 5, 6, 8, 9, 2<sup>nd</sup> and 3<sup>rd</sup> order groups from clade 2) have not been detected since 2008 or earlier; 12 new clades of various orders have been assigned by the WHO/OIE/FAO H5N1 Evolution Working Group in 2011 (WHO/OIE/FAO H5N1 Evolution Working Group, 2011), including the 4<sup>th</sup> order clade 2.3.2.1. The clade 2.3.2 HA genes have diversified to a greater extent than those in other clades; possibly due to its adaptation to different bird species and introduction into multiple geographic regions by poultry trade or migratory birds (WHO/OIE/FAO H5N1 Evolution Working Group, 2012).

The new clade 2.3.2.1 is derived from clade 2.3.2 which is enzootic in China and has been identified in poultry populations as well as in wild birds in many diverse regions outside of China.

#### Geographic distribution and expansion of clade 2.3.2.1

H5N1 clade 2.3.2 viruses were first identified in Hong Kong SAR in a dead wild bird in 2004 and were subsequently confirmed in wild bird outbreaks at Qinghai Lake in China, Uvs Nuur Lake in the Russian Federation and Mongolia during the period 2009–2010 (Hu *et al.*, 2011). These results suggest that migrating wild birds may have played a key role in virus spread. Since then, the virus has been associated with H5N1 HPAI outbreaks

involving poultry and wild birds in Asia and Europe. In Asia, between 2010 and 2012, the H5N1 HPAI virus clade 2.3.2 has spread to 14 countries causing high numbers of outbreaks and unusual levels of mortality in susceptible species, particularly ducks (see Figure 5). Three clusters or groups of clade 2.3.2.1 viruses were identified during 2010–2012 and these are distributed within specific “epizones” or geographic locations in the region (see Figures 3 and 7).

#### South Asia

Between 2006 and 2010, all viruses obtained from HPAI H5N1 outbreaks in South Asia from affected poultry and human cases were virus clade 2.2. During this period, five countries were affected with the virus, including Bangladesh, Nepal, Bhutan, India

Table 1

## H5N1 HPAI clades identified in South Asia during 2006–2012

Country	2006	2007	2008	2009	2010	2011	2012
India	2.2 (Z genotype)	2.2 (Unassigned)	2.2 (EMA3)	2.2 (EMA3)	2.2	2.3.2.1 <sup>a</sup>	2.3.2.1 <sup>b</sup>
Bangladesh		2.2	2.2 <sup>c</sup>	2.2	2.2	2.2, 2.3.2.1, 2.3.4.2	2.3.2.1
Nepal				2.2	2.2	2.3.2	2.3.2.1
Bhutan					2.2.3 (EMA 3)	2.3.2	2.3.2.1

<sup>a</sup> Distinct from Nepal isolates; detected in poultry and wild birds (crows).

<sup>b</sup> In the October to November outbreak in Bangalore (Karnataka) the clade 2.3.2.1 was closely related to the Nepal isolate.

<sup>c</sup> Also in a non-fatal human case.

Table 2

## Viruses isolated from China between 1996 and 2007

Year	HA clades																		Total
	0	1	2.1	2.2	2.3				2.4	2.5	3	4	5	6	7	8	9	Out	
					1	2	3	4											
96-99	9																		9
2000	8												1						9
2001	17										8		1				1	1	28
2002	12	6	1						2			4	2	1		3	2	8	41
2003	4	9	1		1	1			14	4	1	1	3		1	1	6	4	51
2004	4				3	11	1		9	1			16	3	3		13	3	67
2005				19	12	41	5	34	2	0		1			7		9	1	131
2006				4		3		63		2		5			1		1		79
2007																			0
Total	37	15	2	23	16	56	6	97	27	7	9	11	22	4	12	4	32	17	397

Source: Ken Inui, personal communication

and Pakistan. Nepal became the first country in the region to report an incursion of clade 2.3.2 in early 2010 (Nagarajan *et al.*, 2012) followed by India and Bangladesh in early 2011 and Bhutan in early 2012.

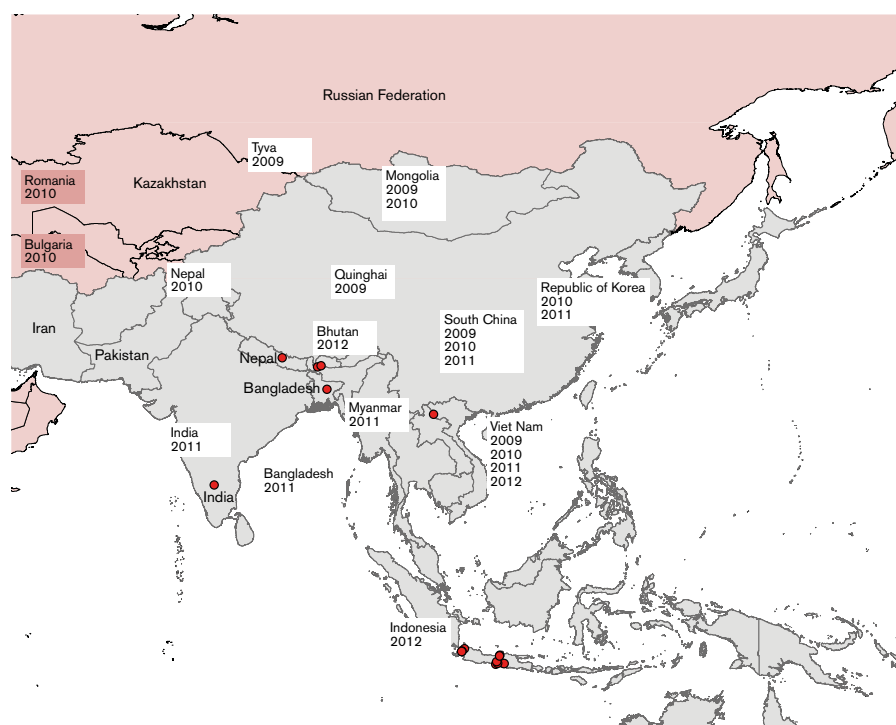
In India, clade 2.3.2.1 was identified from two outbreaks in the east of the country

(Tripura) distinct from that found in Nepal in 2010. The same clade 2.3.2.1 was also isolated from outbreaks in poultry from Odisha state and a large outbreak in crows in Jharkhand state and other parts of India. Between October and November 2012, an outbreak was reported in turkeys, chickens and

ducks on a government farm near Bangalore in Karnataka state. The virus isolated from affected turkeys was identified as 2.3.2.1 which was closely related to the Nepal strain (Ken Inui, personal communication, 10 January 2013). The detail of clades identified from 2006 to 2012 is summarized in Table 1.

Figure 5

## The spread of virus clade 2.3.2.1 in Asia (2009–2012)



Source: Ken Inui

## Southeast Asia

Clade 2.3.2.1 is now widely distributed in Southeast Asia having been confirmed in Vietnam, Myanmar, the Lao People's Democratic Republic and, most recently, in Indonesia during 2012. **Viet Nam** is endemically infected with H5N1 HPAI, and outbreaks were observed in most provinces during 2010–2012. Clade 2.3.2 was first identified in Viet Nam in 2005, and subsequently in 2009, as clade 2.3.2.1 A/Hubei/1/2010-like (HB). Since then it has been found during outbreaks in the north and centre of the country. During 2010–2012, three variants of clade 2.3.2.1 were responsible for outbreaks, namely: 2.3.2.1 A/Hubei/1/2010-like (HB), 2.3.2.1 A/barn swallow/Hong Kong/D10-1161/2010-like (BS) and 2.3.2.1 A/Hong Kong/6841/2010-like (HK), providing evidence for the continued incursion of new viruses into this country or the evolution of existing viruses. Variant A/Hubei/1/2010-like (HB), detected in 2009, was widely distributed and found throughout 2010–2012. Two new variant clades

(2.3.2.1 A/barn swallow/Hong Kong/D10-1161/2010-like (BS) and 2.3.2.1 A/Hong Kong/6841/2010-like (HK)) detected in 2011 and 2012 highlighted concerns about the adequacy of current vaccines to protect poultry against these new strains. Between July and September 2012, variant clade 2.3.2.1 A/Hong Kong/6841/2010-like (HK) was found in association with an increase in outbreak numbers in five provinces located in central and North Viet Nam, and it spread further in South Viet Nam during early 2013.

The repeated detection of new variant clades or new genetic clusters that replace circulating clades in Viet Nam provides some evidence for the existence of a cycle of frequent virus incursions into the north. Viruses introduced in the north spread and circulate locally resulting in the small local epidemics that eventually die out. In addition to clade 2.3.2.1, other clades implicated in outbreaks in Viet Nam during 2010–2012 include clade 1.1 which circulates in the south and has been there since 2004 and clade 2.3.4 which circulated in the north and

south of the country and has been replaced by clade 2.3.2.1 during 2010.

**Myanmar** first reported clade 2.3.2.1 in early 2011 in association with outbreaks in small-scale poultry farms, five years after it first reported H5N1 HPAI outbreaks in 2006. Outbreaks prior to this were caused by 3<sup>rd</sup> order clade 2.3.4. Since the detection of clade 2.3.2.1 A/Hubei/1/2010-like (HB) in 2011, outbreaks have been sporadic with reports occurring during late 2011 and May 2012, all associated with clade 2.3.2.1 A/Hubei/1/2010-like (HB). Viruses isolated from outbreaks during this period were genetically similar to those viruses circulating in Bangladesh, India, Nepal and Bhutan.

**The Lao People's Democratic Republic** has had very few reports of H5N1 HPAI outbreaks and the only reported isolation of clade 2.3.2.1 was from two samples taken during an active surveillance programme conducted in 2011. The two samples yielded virus isolates of clade 2.3.2.1 that were similar to virus isolates from China (2010) and Viet Nam (2011).

## East Asia

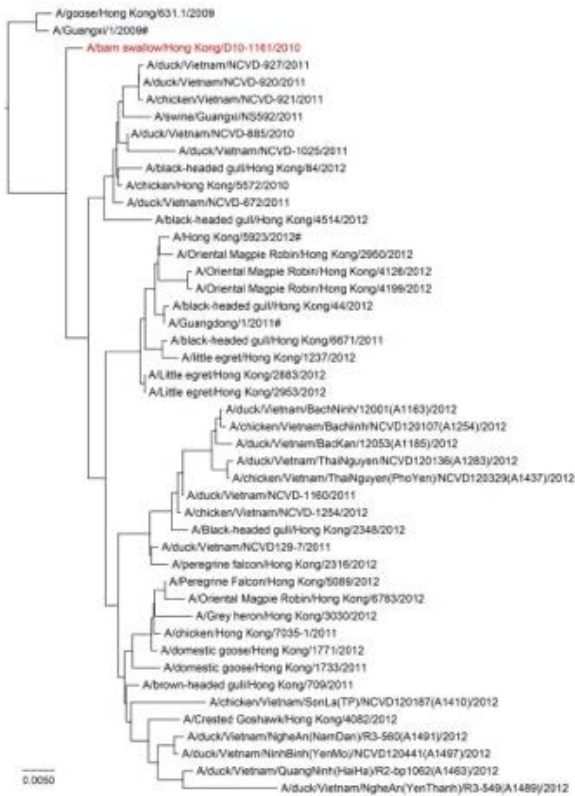
**China** is considered to be the source of clade 2.3.2 viruses given that the clade was first detected in a dead wild bird in Hong Kong SAR in 2004 and has been repeatedly isolated there between 2003 and 2006. Official reports of outbreaks and virus isolations from China have identified the presence of clade 2.3.2.1 and other clades such as clade 7 and 2.3.4 during the period 2010–2012 (Martin *et al.*, 2011; <http://www.moa.gov.cn/zwlml/yjgl/yqfb/>). (See Table 2).

In late 2010 and early 2011, **Japan** and the **Republic of Korea** reported simultaneous outbreaks of H5N1 HPAI in wild birds and poultry, associated with the incursion of genetically similar clade 2.3.2.1 (genetic cluster A/Hong Kong/6841/2010-like (HK)). These viruses were also genetically similar to clade 2.3.2.1 viruses identified in H5N1 HPAI outbreaks in **Nepal** during the same period (see Figure 6). There were no outbreak reports in 2012 implying that the virus has been eliminated from these two countries. In **Mongolia**, all three H5N1 HPAI cases were associated with virus clade 2.3.2 and occurred in wild birds during the early months of 2010 and 2011.

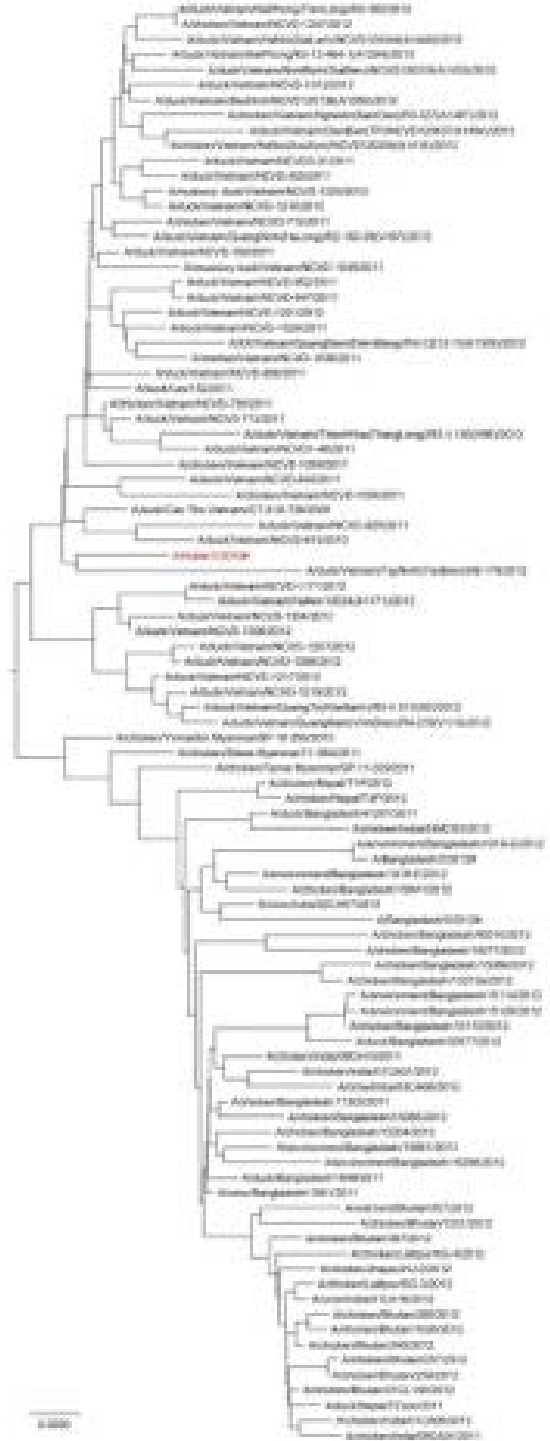
Figure 6

Phylogenetic tree of the three clusters or groups of *clade 2.3.2.1* identified in Asia during 2010–2012

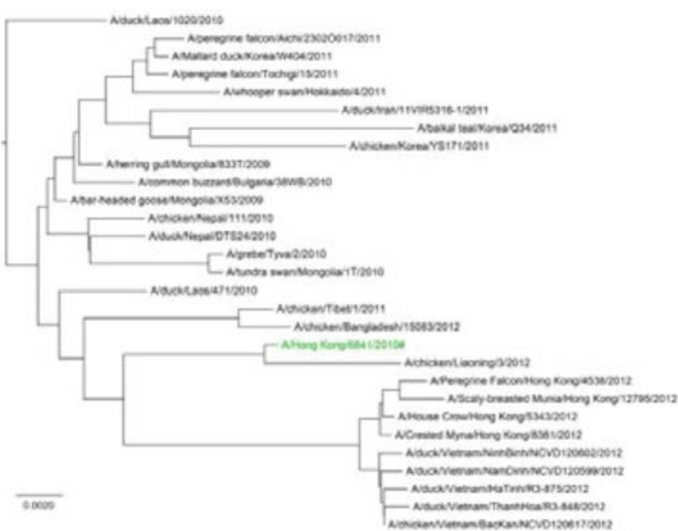
## a) Cluster A/barn swallow/Hong Kong/D10-1161/2010-like (BS)



## b) Cluster A/Hubei/1/2010-like (HB)



## c) Cluster A/Hong Kong/6841/2010-like (HK)





These viruses were similar to those isolated in Nepal (see Figure 5).

### Other regions

In **Europe**, during 2010, three countries reported outbreaks caused by clade 2.3.2 where, in most cases, wild birds were affected. In March 2010, outbreaks were observed simultaneously in **Romania** and **Bulgaria** associated with clade 2.3.2, affecting poultry and wild birds, respectively. Isolates from both countries grouped in the 2010 virus clade 2.3.2.1 were similar to viruses isolated recently from poultry in Nepal in 2010. In June 2010, 367 wild birds were found dead in Ubsu-Nur Lake in the **Russian Federation**. The virus isolates in this outbreak were similar to the 2009–2010 H5N1 isolates responsible for wild bird outbreaks in Mongolia, Tyva and Qinghai Lake in China, providing some evidence as to the possible source of those viruses. All viruses isolated belonged to the A/Hong Kong/6841/2010-like (HK) cluster. This clade has not been isolated from the region since then.

In the **Middle East**, the Islamic Republic of **Iran** reported three outbreaks of H5N1 HPAI in September 2011 in free-ranging poultry caused by virus clade 2.3.2.1 A/Hong Kong/6841/2010-like (HK). A stamping out policy was applied to control the outbreaks. Vaccination against H5N1 HPAI is prohibited in the Islamic Republic of Iran. There have been no more reports of the disease since then. ●

### Risk assessment for the spread of clade 2.3.2.1 in Asia

#### Transmission pathways for clade 2.3.2.1 in Asia

The transmission of influenza viruses may occur via trade in poultry and poultry products, movement of wild migratory birds and trade in wild captive bird species (Olsen *et al.*, 2006; Feare, 2007). The relative importance of each of these routes of transmission of clade 2.3.2.1 in Asia during 2010–2012 is uncertain due to the scarcity of information related to illegal movement of birds, the limited capacity in countries to investigate disease introduction and delays

in reporting disease incursions. Yet, some anecdotal evidence, supported by limited genetic sequence data from virus isolates and outbreak investigations, provide some indication as to the role of each pathway.

#### Role of trade

The role of trade in moving clade 2.3.2 (and subclades 2.3.2.1) viruses across Asia is speculative at best, but the isolation of genetically similar viruses in countries that share borders and where trading links are known to exist may point to the relevance of this route. Clade 2.3.2.1 viruses in Bangladesh and India are genetically similar and it is thought that this clade, initially introduced into Bangladesh in 2010, spilled over into poultry in India during 2011, mainly due to movement of infected poultry. A study by Kilpatrick *et al.* (2007) concluded that the introduction of H5N1 HPAI virus to countries in Asia between 2003 and 2006 was most likely to have occurred through trade in poultry (9 of 21 incursions) and, secondly, by wild migratory birds (3 of 21 incursions). Further sequencing of the virus isolated in Indonesia may provide more insight into its source.

#### Role of wild birds

Support for the role played by wild birds in the spread of clade 2.3.2 across Asia includes the repeated detection of the virus in countries located along migratory pathways of wild birds (e.g Hong Kong SAR, Japan, Mongolia, Republic of Korea, Bangladesh, India and Nepal). For example, Bangladesh, India and Nepal lie along the southwestern end of the East Asian-Australasian Flyway – migratory corridors for birds extending to Russia at its northern limit. Though Indonesia lies further south on the same Flyway, this is the first time that clade 2.3.2 has appeared in the country, despite the presence of the virus in other countries along this pathway. This result might imply the lack of importance of wild birds in the new incursion of this clade into Indonesia, a possible change in wild bird behaviours or it might highlight the role played by poultry movement from affected countries in the introduction of the disease. Additionally, Indonesia has never reported outbreaks of H5N1 HPAI in wild birds.

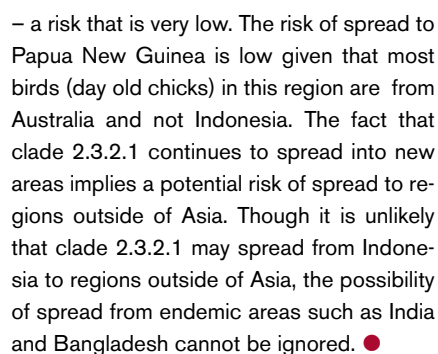
### Risk management

Numerous commercial vaccines are available against H5 influenza viruses many of which do not provide adequate protection against virus clade 2.3.2.1 (see list of commercially available vaccines at: <ftp://ftp.fao.org/docrep/fao/011/ai326e/ai326e00.pdf>). Most vaccines available in Indonesia are classical inactivated vaccines locally manufactured from 2.1.3 clade viruses. Initial experimental trials conducted in Indonesia have shown absence or insufficient cross-protection of these Indonesian vaccines against the newly introduced 2.3.2.1 clade viruses. This result implies that bivalent vaccines will be required to ensure a good immunity against circulating strains, in the likely situation that the two clades will coexist in Indonesia. Production of bivalent vaccines poses challenges in terms of capacity and cost since two different antigens must be produced to be incorporated into the same vaccine. The introduction of clade 2.3.2.1 in Indonesia, if it persists in the country, will, therefore, have a severe impact on the effectiveness of vaccination in Indonesia. It may even have a competitive advantage over clade 2.1.3 viruses against which there is immunity in part of the poultry population.

### Areas and bird populations at risk

The poultry population in Indonesia and surrounding countries could be considered a naïve population and, therefore, highly susceptible to clade 2.3.2.1 and other emergent H5N1 clades. Clade 2.3.2.1 is expected to spread widely in Indonesia, but Kalimantan remains at high risk unless national veterinary authorities are able to stop the spread by implementing biosecurity measures effectively. Continued circulation of this new virus within poultry populations in Indonesia is likely given this country's recent experience with clade 2.1 and the presence of factors such as dense poultry populations, live bird market trading and ineffective use of vaccines, all of which provide conditions for influenza viruses to persist. The risk posed to countries like Australia and New Zealand is dependent on the ability of the virus to move from Indonesia through wild birds and trade

Phylogentic tree showing the relationship between H5N1 clade 2.3.2.1 viruses circulating in Asia 2010–2012



Measures implemented to control avian influenza outbreaks are based on achieving early disease detection and control to reduce virus transmission with the final goal of elimination in the poultry sector based on surveillance, targeted culling and disposal, biosecurity and vaccination.

- reactivate surveillance in wild birds by joint intervention between veterinary services and the Ministry of Forestry;
- characterize virus isolates from chickens in proximity to duck outbreaks in order to elucidate the transmission mechanisms and epidemiology of the clade 2.3.2.1;
- assess immediately the protection produced by local H5N1 vaccines against the newly introduced clade;
- consider importing Re-6 vaccine (produced in China) as an alternative emergency measure until such time as Indonesian vaccine manufacturers can develop an effective vaccine against clade 2.3.2.1;
- characterize further virus isolates from outbreaks across the affected islands

- assess the sensitivity of current PCR tests used in Indonesia to detect clade 2.3.2.1 with the support of FAO/OIE Reference Centres (e.g. Australian Animal Health Laboratory (AAHL));
- share results from HA/HI tests carried out on new clade isolates in order to determine whether these tests can be used in pre-screening to detect the new clade;
- obtain phylogenetic tree maps of viruses circulating in Asia and share with laboratory scientists who are genetically mapping the Indonesian viruses. Placing Indonesian viruses in this tree map may point to the possible source of the introduced clade 2.3.2.1 virus.

Given the continued risk of the emergence of new influenza (H5N1) virus clades, it

is important that endemic countries in South, Southeast and East Asia follow some general recommendations. They should:

- implement appropriate control measures in the face of an HPAI outbreak, which should include: quarantine, humane culling and depopulation, compensation, poultry movement management and increased biosecurity (cleaning and disinfection of facilities and transport vehicles) at farm level and in live bird markets;
- encourage outbreak reporting by stakeholders to understand the extent of the outbreaks and ensure adequate resources are available for a rapid response;
- investigate disease outbreaks, submitting appropriate samples to national laboratories (to be confirmed at an FAO or OIE reference laboratory) to identify and ensure that new emerging virus clades are quickly detected among endemic clades;
- monitor endemic field strains/clades routinely to understand when new strains have emerged;
- conduct vaccine efficacy trials against emerging strains of virus routinely to ensure available vaccines provide adequate protection.

The use of vaccines to control outbreaks of avian influenza should be part of a comprehensive control strategy that includes biosecurity, quarantine, surveillance and diagnostics, education and elimination of infected poultry (Swayne, 2011). Vaccines should:

- be used strategically, based on the epidemiological situation, risk analyses, commercial and economic considerations, and as a result of directives from the national veterinary services;
- be used in conjunction with epidemiological surveillance and biosecurity measures to ensure containment and elimination of circulating field viruses in poultry production systems;
- be selected based on the effectiveness of protection against circulating



Farm in the town of Lendak, Medical Veteriner District of Kulon Progo Yogyakarta.

© AFP Photo/Arif Ariadi

- virus strains, with potency and safety established;
- meet or exceed OIE international standards, in the case of imported vaccines;
- be integrated with a planned exit strategy developed to include surveillance and immediate reporting to authorities of any suspect case of viral activity.

In order to reduce the risk of emergence and the cycle of transmission of these new clades, it is important that countries adopt biosecurity practices along the poultry production chain, including principles of segregation, cleaning and disinfection.

- To prevent the introduction of the virus, including through channels of commerce and trade, countries should:
  - develop a biosecurity plan tailored to the relevant premises, trade channels and markets;
  - quarantine all birds, whether new or returning, that enter the premises;
  - clean and disinfect cages, egg crates and vehicles before and after use;
  - use one set of outer clothing, when handling poultry on a farm which is not to be used off the farm;
  - protect poultry from coming into contact with wild birds, where possible.
- To eradicate or control an outbreak as soon as it is detected, countries should:

- use depopulation and humane culling, with proper and biosecure disposal;
- clean and disinfect exposed premises, cages, equipment, vehicles;
- quarantine movement into and out of the affected premises;
- trace movements of poultry, and quarantine and assess potentially exposed poultry, farms, markets, trade channels, products, cages and vehicles.

- To reduce the consequences once the virus is present, countries should:
  - maintain biosecurity measures to minimize movement of the virus within and beyond the affected premises;
  - ensure that vaccination teams exercise biosecurity when vaccines are used on a farm to minimize the chance that they act as fomites, enabling the virus to move from one house or farm to another.

The links that exist between countries through trade favour the exchange of viruses across borders, so a regional approach based on common surveillance, the sharing of laboratories and information is encouraged, along with agreed upon regional contingency plans.

FAO encourages countries to report H5N1 HPAI outbreaks (glews@fao.org) and offers assistance to countries in the shipment of samples for transboundary animal disease (TAD) diagnostic testing to an

OIE or FAO reference centre. Contact [empres-shippingservice@fao.org](mailto:empres-shippingservice@fao.org) for information prior to sample shipment and note that sending samples out of a country requires an export permit from the Chief Veterinary Office of the country. ●

### Further details on control measures are available from:

*Good emergency management practice: the essentials.* FAO Animal Production and Health Paper, No. 11 (available at <http://www.fao.org/docrep/014/ba0137e/ba0137e00.pdf>).

*Good emergency management practices: SOP for HPAI response* (available at <http://www.fao.org/docrep/015/i2364e/i2364e00.pdf>).

*Preparing for highly pathogenic avian influenza.* FAO Animal Production and Health Manual, No. 3 Revised Edition (available at <ftp://ftp.fao.org/docrep/fao/012/i0808e/i0808e.pdf>).

*Wild bird highly pathogenic avian influenza surveillance - sample collection from healthy, sick and dead birds.* FAO Animal Production and Health Manual, No. 4 (available at <ftp://ftp.fao.org/docrep/fao/010/a0960e/a0960e00.pdf>).

*Biosecurity for highly pathogenic avian influenza - issues and options.* FAO Animal Production and Health Paper, No. 165 (available at <ftp://ftp.fao.org/docrep/fao/011/i0359e/i0359e00.pdf>).

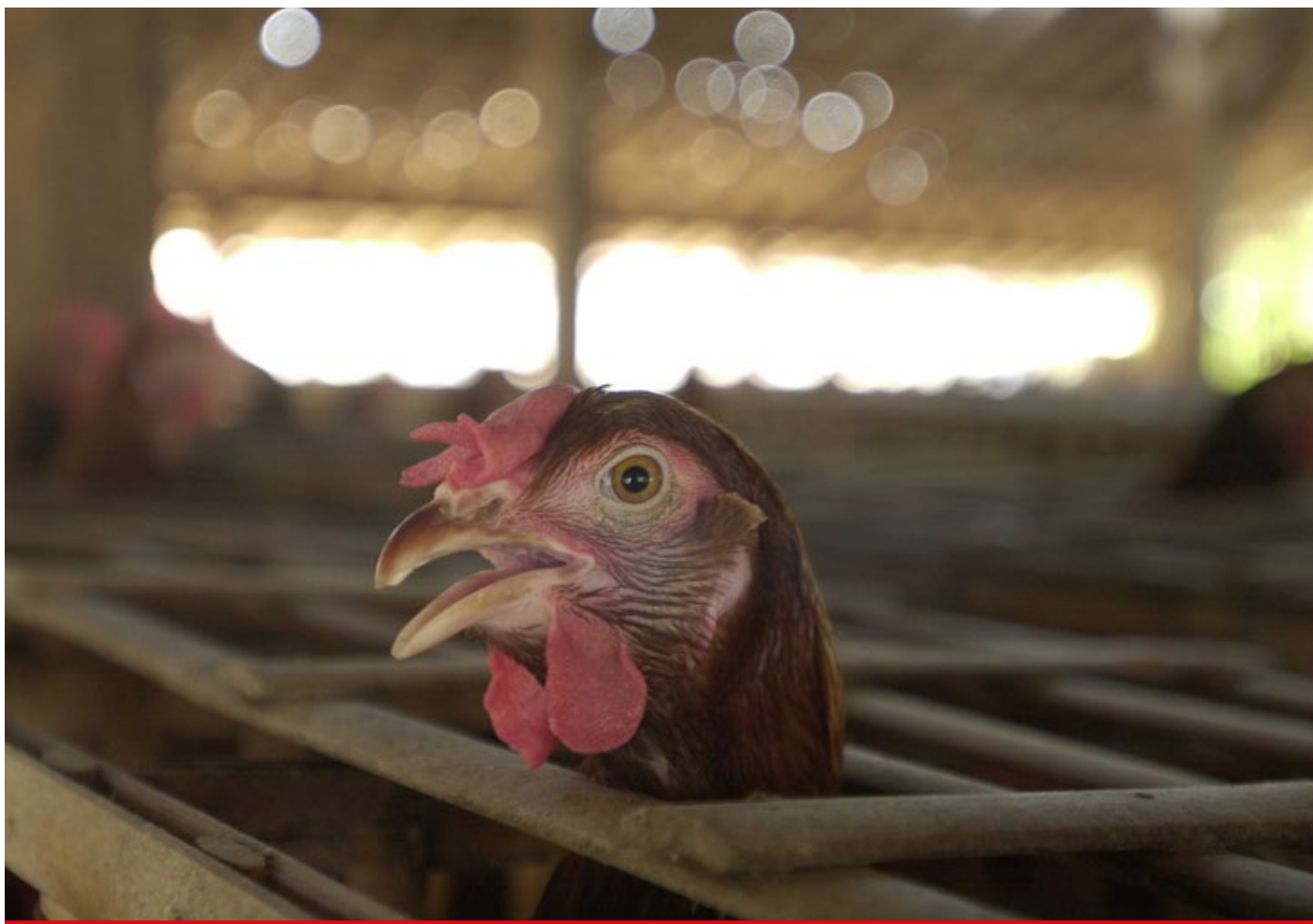
*Understanding avian influenza* (available at [http://www.fao.org/avianflu/documents/key\\_ai/key\\_book\\_ch2.htm](http://www.fao.org/avianflu/documents/key_ai/key_book_ch2.htm)).

*Approaches to controlling, preventing and eliminating H5N1 highly pathogenic avian influenza in endemic countries* (available at <http://www.fao.org/docrep/014/i2150e/i2150e.pdf>).

## References

- Campitelli, L., Fabiani, C., Puzelli, L., Fioretti, E., Foni, E., De Marco, A., Krauss, S., Webster, R. & Donatelli, I. 2002. H3N2 influenza viruses from domestic chickens in Italy: an increasing role for chickens in the ecology of influenza? *Journal of General Virology*, 83: 413–420.
- Duan, L., Bahl, J., Smith, G.J., Wang, J., Vijaykrishna, D., Zhang, L.J., Zhange, J.X., Li, K.S., Fan, X.H., Cheung, C.L., Huang, K., Poon, L.L., Shortridge, K.F., Webster, R.G., Peiris, J.S., Chen, H. & Guan, Y. 2008. The development and genetic diversity of H5N1 influenza virus in China, 1996–2006. *Virology*, 380: 243–254. (available at <http://www.ncbi.nlm.nih.gov/pubmed/18774155>).
- Feare, C. 2007. The role of wild birds in the spread of HPAI H5N1. *Avian Diseases*, 51: 440–447.
- Guan, Y., Poon, L.L.M., Cheung, C.Y., Ellis, T.M., Lim, W., Lipatov, A.S., Chan, K.H., Sturm-Ramirez, K.M., Cheung, C.L., Leung, Y.H.C., Yuen, K.Y., Webster, R.G. & Peiris, J.S.M. 2004. H5N1 influenza: a protean pandemic threat. *Proceedings of the National Academy of Sciences of the United States of America*, 101 (21): 8156–8161.
- Horimoto, T. & Kawaoka, Y. 2001. Pandemic threat posed by avian influenza A Viruses. *Clinical Microbiological Review*, 14: 129–149.
- Kilpatrick, A., Chmura, A., Gibbons, D., Fleischer, R., Marra, P. & Daszak, P. 2006. Predicting the global spread of H5N1 avian influenza. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 19368–19373. (available at <http://www.pnas.org/content/103/51/19368.abstract>).
- Kou, Z., Li, Y., Yin, Z., Guo, S., Wang, M., Gao, X., Li, P., Tang, L., Jiang, P., Luo, Z., Xin, Z., Ding, C., He, Y., Ren, Z., Cui, P., Zhao, H., Zhang, Z., Tang, S., Yan, B., Lei, F. & Li, T. 2009. The survey of H5N1 flu virus in wild birds in 14 provinces of China from 2004 to 2007. *PLoS ONE*, 4(9). (available at <http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0006926>).
- Martin, V., Pfeiffer D.U., Zhou, X., Xiao, X., Prosser, D.J., Fusheng, G. & Gilbert, M. 2011. Spatial distribution and risk factors of highly pathogenic avian influenza (HPAI) H5N1 in China. *PLoS Pathogens*, 7(3). (available at <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1001308>).
- McLeod, A., Morgan, N., Prakash, A. & Hinrichs, J. 2004. *Economic and social impacts of avian influenza*. FAO technical report. (available at: <http://www.fao.org/docs/eims/upload//211939/Economic-and-social-impacts-of-avian-influenza-Geneva.pdf>).
- Nagarajan, S., Tosh, C., Smith, D.K., Peiris, J.S.M., Murugkar, H.V., Sridevi, R., Kumar, M., Katare, M., Jain, R., Syed, Z., Behera, P., Cheung, C.L., Khandia, R., Tripathi, S., Guan, Y. & Dubey, S.C. 2012. Avian Influenza (H5N1) virus of clade 2.3.2 in domestic poultry in India. *PLoS ONE* 7(2). e31844.doi:10.1371/journal.pone.031844.
- Olsen, B., Munster, V., Wallensten, A., Waldenstrom, J., Osterhaus, A. & Fouchier, R. 2006. Global patterns of influenza A virus in wild birds. *Science*, 312, 384–388.
- Peiris, J.S.M., de Jong, M.D. & Guan, Y. 2007. Avian influenza virus (H5N1): a threat to human health. *Clinical Microbiology Review*, 20 (2): 243–267.
- WHO/OIE/FAO H5N1 Evolution Working Group. 2011. *Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness*. Sept 2011. (available at [http://www.who.int/influenza/resources/documents/2011\\_09\\_h5\\_h9\\_vaccinevirusupdate.pdf](http://www.who.int/influenza/resources/documents/2011_09_h5_h9_vaccinevirusupdate.pdf)).
- WHO/OIE/FAO H5N1 Evolution Working Group. 2012. *Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness*. Sept. 2012. (available at [http://www.who.int/influenza/vaccines/virus/201209\\_h5h7h9\\_vaccinevirusupdate.pdf](http://www.who.int/influenza/vaccines/virus/201209_h5h7h9_vaccinevirusupdate.pdf)).
- Webster, R.G., Peiris, M., Chen, H. & Guan, Y. 2006. H5N1 outbreaks and enzootic influenza. *Emerg Infect Dis*, 12 (1): 3–8.
- Sims, L.D., Domenech, J., Benigno, C., Kahn, S., Kamata, A., Lubroth, J., Martin, V. & Roeder, P. 2005. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Veterinary Record*, 157(6): 159–64.
- Zhao, Z.M., Shortridge, K.F., Garcia, M., Guan, Y. & Wan, X.F. 2008. Genotypic diversity of H5N1 highly pathogenic avian influenza viruses. *Journal of General Virology*, 89: 2182–2193.





## focus on CONTACT

The Emergency Prevention System (EMPRES) is a FAO programme, founded in 1994, with the goal of enhancing world food security, fighting transboundary animal and plant pests and diseases, and reducing the adverse impact of food safety threats. EMPRES-Animal Health is the component dealing with the prevention and control of transboundary animal diseases.

To subscribe to this bulletin or to ask for information about EMPRES-Animal Health send an e-mail to [empres-livestock@fao.org](mailto:empres-livestock@fao.org) or fax to **+39 06 57053023**

For more information visit us at:

<http://www.fao.org/ag/empres.html>

EMPRES-Animal Health can assist countries in the shipment of samples for diagnostic testing of transboundary animal diseases (TADs) at FAO reference laboratories and centres. Please contact **EMPRES-Shipping-Service@fao.org** for information prior to sampling or shipment. Please note that sending samples out of a country requires an export permit from the Chief Veterinarian's Office of the country and an import permit from the receiving country.



### Recommended citation

**FAO.** 2013. Update on the continuous spread and expansion of H5N1 highly pathogenic avian influenza: Clade 2.3.2.1 in Asia (2010–2012), *FOCUS ON*, No. 6, November 2013. Rome.

**Main cover photo:** © FAO/John Edwards

**Back cover photo:** © AFP Photo/Arif Ariadi

## COPYRIGHT NOTICE AND DISCLAIMER

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether

or not these have been patented, does not imply that these have been endorsed or recommended by FAO in preference to others of a similar nature that are not mentioned. The views expressed in this information product are those of the author(s) and do not necessarily reflect the views of FAO.

All rights reserved. FAO encourages the reproduction and dissemination of material in this information product. Non-commercial uses will be authorized free of charge, upon request. Reproduction for resale or other commercial

purposes, including educational purposes, may incur fees. Applications for permission to reproduce or disseminate FAO copyright materials, and all queries concerning rights and licences, should be addressed by e-mail to [copyright@fao.org](mailto:copyright@fao.org) or to the Chief, Publishing Policy and Support Branch, Office of Knowledge Exchange, Research and Extension, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy.

© FAO 2013