



## Avian influenza A(H5N6): the latest addition to emerging zoonotic avian influenza threats in East and Southeast Asia



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### Context of new emerging subtypes of Avian Influenza viruses in eastern Asia

The first reported appearance of a global zoonotic influenza of avian origin dates from 1996, when an outbreak of H5N1 highly pathogenic avian influenza (HPAI) occurred on a goose farm in Guangdong Province, China [Guan et Smith, 2013]. One year later, the H5N1 HPAI virus was recognised as a zoonotic agent with 18 human cases identified in

Hong Kong Special Administrative Region (SAR), including six human fatalities [WHO]. A program of massive culling of poultry in the autonomous region was necessary to put an end to the epidemic [De Jong *et al*, 1998]. A few years later the H5N1 HPAI re-emerged in Asia on a much larger scale. Two human cases were first reported in Hong Kong SAR in February 2003 [Peiris *et al*, 2004], before the Republic of Korea reported several poultry outbreaks in December 2003. Between late 2003 and early 2004 H5N1 HPAI expanded rapidly throughout East and Southeast Asia, with more than 1,300 outbreaks in Cambodia, China, Lao People’s Democratic Republic, the Republic of Korea, Thailand and Viet Nam reported in January 2004 alone [EMPRES-i]. By mid-2006, the virus had spread to Europe, Middle East and Africa, affecting over 60 countries and territories. Most countries succeeded in eliminating the disease from the poultry sector with few endemic hot spots remaining in the Indo-Gangetic Plain and the Greater Mekong subregions, China, Indonesia, and Egypt [EMPRES-i]. The nature of the poultry production systems including poor biosecurity and inadequate veterinary services confounded efforts to control the disease effectively, and it is unlikely that the disease will be eliminated in these countries in the foreseeable future without major changes in production and marketing practices to improve hygiene, inspection and compliance.

A diverse viral gene pool now exists in eastern Asia due to co-circulation of many different avian influenza (AI) viruses. Following genetic reassortment events between viruses or mutation of viral genes, novel virus strains continue to emerge in the region with unpredictable consequences for animal and human health. Recently, the detection of such new reassortants has become more frequent.

Since early 2013 several new avian influenza virus strains have been reported in East and Southeast Asia, threatening both poultry-dependant livelihoods and human health. In March 2013, avian influenza A(H7N9) emerged in China causing a total of 456 human cases, and claiming 172 lives as of 05 November 2014. The virus is of low pathogenicity in poultry (no clinical signs are observed in infected birds) but can cause lethal pulmonary infection in mammals without prior trans-species adaptation [Can *et al*, 2014]. Subsequently, H10N8 was reported in December 2013, causing three human cases in China, including two fatalities, while H5N8 emerged the month after and was the cause of at least 40 poultry outbreaks, mainly in the Republic of Korea but also in Japan and China [OIE; EMPRES-i]. This was followed by the first reports from China and Lao People’s Democratic Republic in early May 2014, describing outbreaks in poultry caused by a highly pathogenic H5N6. More recently, the finding of a H5N3 subtype was reported for the first time on 24 October 2014 from a live bird market in Changsha, Hunan, China [OIE]. Concerns grow as the rate of emergence of new sub-types increases, creating new potential threats and a need for continuous vigilance and monitoring (Table 1 and Figure 1).

### Emergence of H5N6

Low pathogenic viruses belonging to the H5N6 subtype have already occurred in the past. Found mainly in duck but also in environmental samples, H5N6 viruses had already been detected in various areas of the world, including Wisconsin, the United States of America in 1975 [GenBank acc. U79451], Germany in 1984 [GenBank],

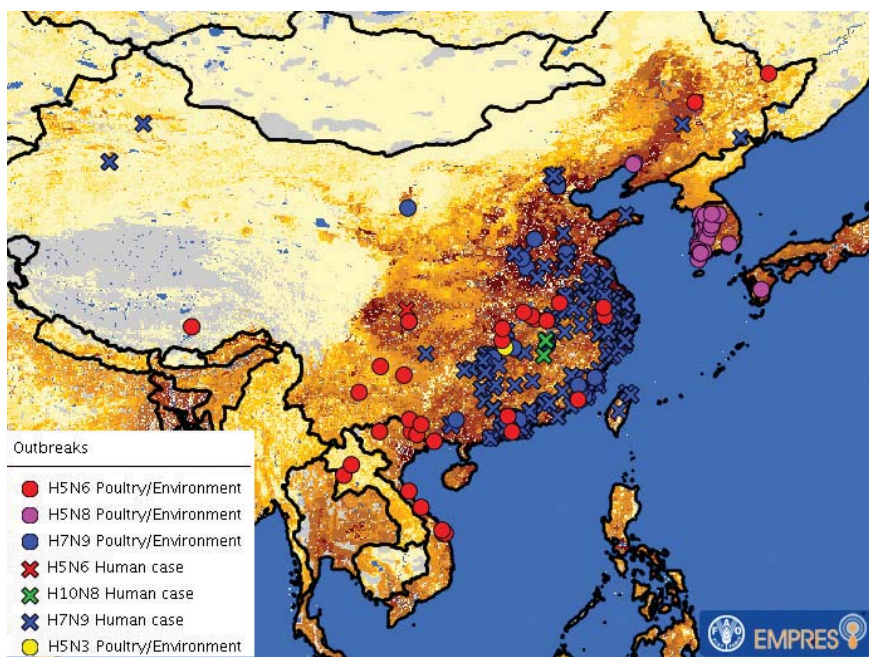
**Table 1: New avian influenza virus types and sub-types detected in East and Southeast Asia since early 2013** (as of 05 November 2014).

Type and Sub-type	Location	Human cases	Clinical signs in poultry	Poultry outbreaks	First report
H7N9	China	455	No	*	March 2013
H10N8	China	3	?	?	December 2013
H5N8	China, Korea, Japan	0	Yes	42	January 2014
H5N6	China, Lao PDR, Viet Nam	1	Yes	34	May 2014
H5N3	China	0	Yes	1	October 2014

\*In the absence of clinical signs in poultry the term outbreak cannot be used. However the virus has been found in 132 out of over 600,000 samples collected by the Chinese Ministry of Agriculture from poultry or the environment and 63 environmental samples collected during trace-back surveillance of human cases in China between April 2013 and September 2014. Remarkably, peer-reviewed publications reported 326 positive virological samples out of 22,197 [1, 2, 3, 4, 5, 6, 7, 8].

**Figure 1. Overview of avian influenza virus strains circulating in East and Southeast Asia that emerged since 2013**

Data: EMPRES-i\*



\* Data in EMPRES-i is derived from numerous sources: FAO representatives or country missions, FAO reports, World Organisation for Animal Health (OIE), official government sources, the European Commission (EC), peer-reviewed publications, FAO reference centres, laboratories and FAO collaborators.

Sweden in 2002 [GenBank acc. AY995892], and California, the United States of America in 2013 [GenBank acc. CY177425]. These outbreaks however had little to no impact on the poultry industry and no implications for public health.

Unlike its predecessors, the recently emerged Asian H5N6 strain is a highly pathogenic AI virus, which causes severe clinical signs in poultry and associated mortality. The first poultry outbreak caused by H5N6 was reported in Nanchong City, Sichuan Province, China, on 4 May 2014 [OIE], after confirmation by both reverse transcription polymerase chain reaction (RT-PCR) and virus isolation at the Harbin Veterinary Research Institute (OIE/FAO Reference Centre). Genetic sequences demonstrate that the virus was already

present in China several months prior to notification, though no outbreak was officially reported [OIE]; the virus had been isolated from an environmental sample collected on 20 December 2013 in a live poultry market in Zhenjiang, Jiangsu Province [Qi *et al*, 2014] and from a duck in Guangdong Province in March 2014 [GenBank acc. KJ754145].

Shortly after, Lao People's Democratic Republic and Viet Nam both reported two and seven H5N6 poultry outbreaks, respectively, with initial outbreak observation in March 2014 in Lao People's Democratic Republic and in April 2014 in Viet Nam (Table 2). On 24 October 2014, China notified OIE of another 24 positive findings in poultry or the environment, reported in 12 different provinces from Heilongjiang in the northeast of China down to Tibet in the southwest.

This suggests that H5N6 is already fairly widespread in eastern Asia (Figure 1).

According to official information, for each outbreak, contingency measures such as stamping out, movement control, and disinfection of infected premises were implemented [OIE, 2014].

## H5N6 in Humans

Only one human H5N6 infection has been detected to date. This case, a 49-year-old male farmer from Sichuan Province, China, associated to the first poultry outbreak, was reported on 6 May 2014 and died from severe pneumonia a few days after hospitalization. Close contacts of the patient were placed under medical observation without additional human cases detected [HFPC Sichuan, 2014].

Given the current evidence, and the fact that despite the virus being detected in poultry across a fairly widespread geographical area in eastern Asia, only one human case was reported, WHO considers that H5N6 is not likely to be easily transmissible from birds to humans.

## Genetic characteristics of H5N6 viruses

Analysis of the eight genome segments of H5N6 viruses from Viet Nam and Lao People's Democratic Republic indicated that these viruses are genetically similar. The hemagglutinin (HA) gene belongs to a specific H5 clade 2.3.4.4 group<sup>1</sup>. The recently isolated H5N1 HPAI viruses from live bird markets in Viet Nam and the H5N8 viruses detected in Republic of Korea and Japan also belong to this genetic group<sup>2</sup>. The neuraminidase (NA) gene is closely related to

<sup>1</sup> While the HA genes from viruses in this emerging virus group meet the criteria for novel clade designation, a formal nomenclature has not yet been adopted. 2.3.4.4 is used as a provisional nomenclature only.

<sup>2</sup> It will be interesting to ascertain if the H5 from the H5N3 that was recently detected in a Chinese live bird market also belongs to the same clade. At the time of writing its genetic information was not available.

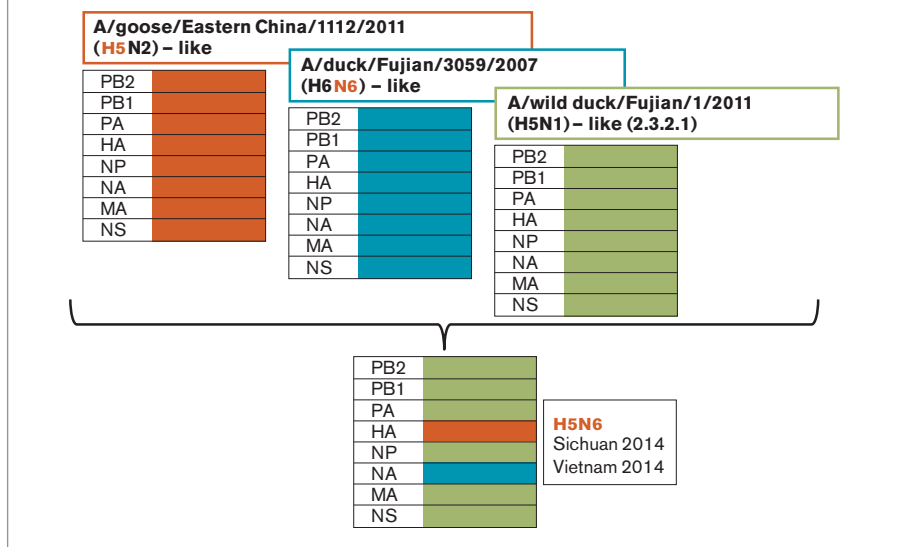
**Table 2. Reports of poultry outbreaks caused by H5N6 HPAI in East and Southeast Asia, sorted by sample collection date as reported to the OIE (as of 05 November 2014) [OIE, 2014].**

Collection Date	Reporting Date	Location	Species	Unit	Susceptible	Cases	Deaths
13/03/14	05/05/14	Xayabury LAO PDR	Chicken, Duck	Village	5 142	457	457
22/04/14	12/08/14	Lang Son VIET NAM	Chicken	Village	80	40	15
23/04/14	04/05/14	Sichuan CHINA	Chicken	Farm	1 338	1	0
25/06/14	12/08/14	Ha Tinh VIET NAM	Duck	Village	1900	950	0
14/07/14	15/09/14	Luangprabang, LAO PDR	Chicken, Duck	Farm	200	2	2
11/08/14	22/08/14	Lao Cai VIET NAM	Pheasant	Village	558	498	498
15/08/14	04/09/14	Quang Tri VIET NAM	Duck	Village	1 550	350	350
23/08/14	01/09/14	Heilongjiang CHINA	Goose	Farm	86 674	20 550	17 790
23/08/14	04/09/14	Quang Ngai VIET NAM	Duck	Village	1 100	200	200
12/09/14	24/10/14	24 outbreaks in 12 provinces in China	Duck, Chicken, Environment				
13/09/14	20/09/14	Quang Ngai VIET NAM	Duck	Backyard	1 000	1 000	300
14/09/14	20/09/14	Quang Nam VIET NAM	Duck	Backyard	2 000	2 000	600

that of a group of H6N6 viruses circulating in domestic ducks in Southern China. Internal genes show a high similarity to those of the H5 virus clade 2.3.2.1.B H5N1 virus A/wild duck/Fujian/1//2011. Thus H5N6 is likely to be a triple-reassortant virus (Figure 1). Genetic features of the novel A(H5N6) viruses isolated thus far from the human case or poultry outbreaks can be summarized as follows:

- **High pathogenicity for poultry:** Novel A(H5N6) viruses have the polybasic HA cleavage sequence, PLRERRRKR/GLF common to clade 2.3.4 HPAI viruses. This indicates that they are highly pathogenic avian influenza viruses, capable of causing high mortalities in poultry.
- **Preference for avian-like receptor binding:** The amino acid characteristics of the hemagglutinin of these novel H5N6 viruses show a predicted preference for avian-like  $\alpha$ 2,3-linked sialic acid receptors. This means that H5N6 has currently more avian than mammalian-like receptor affinity, contrary to the recently emerged H7N9 LPAI in China. However these viruses do contain a Thr156Ala substitution, which disrupts N-glycosylation at position 154, potentially leading to enhanced receptor-binding affinity to mammalian receptors [Herfst *et al*, 2012].
- **Adaptation to terrestrial poultry:** The NA gene from H5N6 contains an 11-amino acid deletion in the NA stalk region (positions 59–69; N6 numbering) as previously found in A(H6N6) viruses isolated from domestic ducks in Guangdong Province in China [Herfst *et al*, 2012].
- **No evidence of mammalian host adaptation:** The PB2 E627K mutation

**Figure 2. Hypothetical source of origin of the H5N6 triple reassortant based on closest relationship to known ancestral viruses in GenBank**



linked to mammalian host adaptation was not present in the A(H5N6) viruses isolated so far.

- **Sensitivity to Oseltamivir (Tamiflu®):** No known N1 and N2 neuraminidase inhibitor resistance markers present in known A(H5N6) viruses, thus indicating that these viruses are sensitive to oseltamivir [Boltz *et al*, 2010].

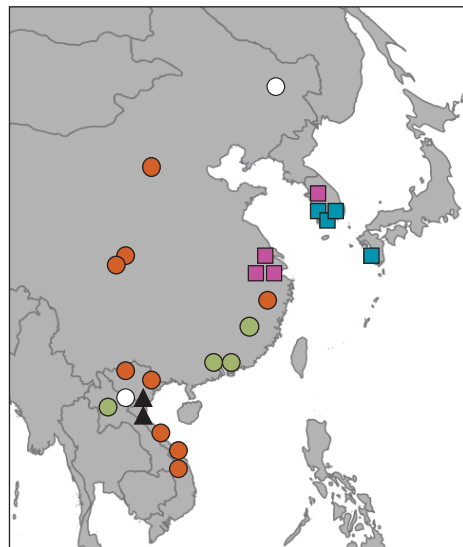
### Phylogenetic analysis of H5N6, H5N8 and H5N1 viruses

When analyzing the HA gene of the emerging clade 2.3.4.4 viruses (H5N1, H5N6, and H5N8), three distinct groups are found

(Figure 3). A first group consists of the emerging H5N8 viruses isolated in 2013–2014 in China and the Republic of Korea (pink squares). These viruses show a close genetic relationship to some older H5N1/H5N2 HPAI viruses isolated in ducks and wild birds in China in 2010–2011. A second genetic cluster contains the newly isolated H5N1 HPAI from live bird markets in Viet Nam (black triangles), H5N8 viruses isolated in the Republic of Korea and Japan, and the H5N6 viruses isolated in Lao People’s Democratic Republic and in Guangdong Province, China (green circles). The third group in this 2.3.4.4 clade is formed of Vietnamese and Chinese H5N6 viruses (red circles). It shows that all five Vietnamese cases are closely related to each other, and are also closely related to

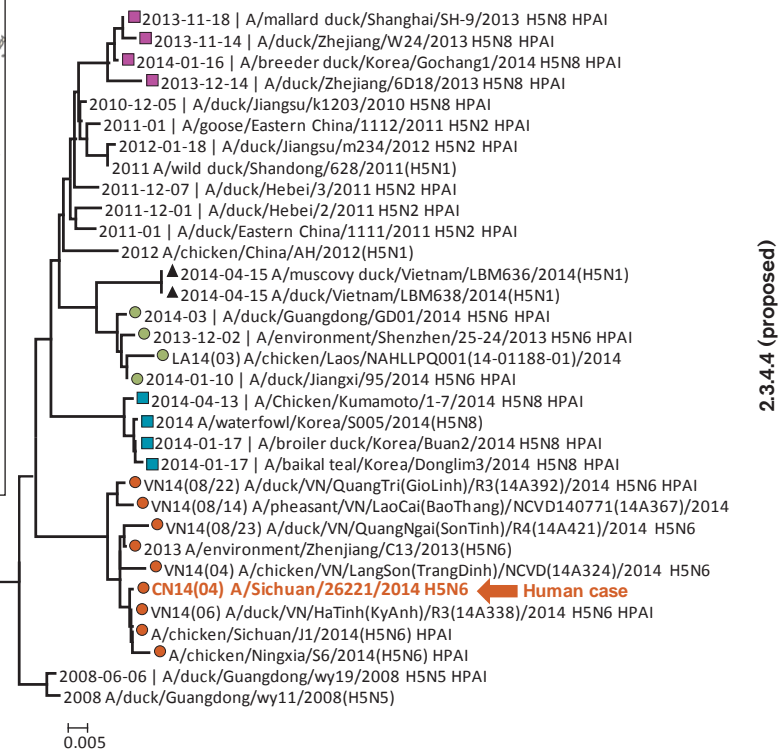


**Figure 3. Genetic similarity of H5 clade 2.3.4.4 viruses in East and Southeast Asia**



	H5N6	Sichuan - like
	H5N6	Laos - like
	H5N6	Sequence not available yet
	H5N8	Korea /Gochang - like
	H5N8	Japan/Kumamoto - like
	H5N1	Vietnam (new 04/2014)

**Emergence and spread of HPAI H5N6, H5N8 and H5N1 with HA gene clade 2.3.4.4 in Asia 2013-2014**



H5N6 virus (A/Sichuan/2622/2014) detected in a human case in April 2014 in Sichuan Province, China as well as from isolates from chickens from Sichuan and NingXia Provinces. The phylogenetic tree shows that the Vietnamese/Chinese H5N6 virus group is slightly different from H5N6 virus detected in March 2014 in Lao People’s Democratic Republic and from a duck virus isolated in Guangdong Province, China, thus indicating an already existing genetic diversity within this newly emerging virus clade.

## Perspectives and recommendations

The epidemiological and genetic analysis described above show that the virus is still more adapted to avian-type than human-type respiratory receptors. However, with winter season approaching in eastern Asia, the prevalence of the virus in poultry is expected to rise, increasing both chances for human exposure and opportunities for mutation or reassortment, especially considering the location of circulation of this virus: in endemic regions with H5N1, H9N2 and H7N9 for China. The latter could change the viruses’ transmissibility to humans. H5N6 therefore remains a public health threat, which requires close monitoring in the same way as for H5N1 HPAI and H7N9.

The possibility exists that wild birds could become infected and spread these viruses

to other countries or continents. Migratory birds, which have played a key role in the introduction of H5N1 to Europe and Africa [Kilpatrick *et al*, 2006] and of H5N8 to the Republic of Korea [Jeong *et al*, 2014], could spread the viruses to other countries or continents. Research is required to better understand the role migratory birds play in the epidemiology of these novel viruses and related risks.

FAO is coordinating the evaluation of AI poultry vaccines currently in use in Viet Nam. Preliminary results are encouraging as two out of the three vaccines tested showed a drastic reduction of disease in chickens [FAO unpublished data]. One of FAO’s Reference Centres, the Australian Animal Health Laboratory (AAHL) in Geelong, has established that current avian influenza tests can detect H5N6 effectively. In addition, new, more specific tests are under development. Diagnostic protocols, soon available on the OIE/FAO Network of Expertise on Animal Influenza website [OFFLU], are also under review to enhance diagnostic preparedness in the region.

With its own funding and that of a grant received from United States Agency for International Development, FAO is also assisting countries in implementing AI surveillance to enable early detection of incursion or spread of H7N9, H5N6 and other avian influenza viruses. Countries with ongoing H5N1 HPAI surveillance are strongly recommended to ensure that all H5 positive,

N1 negative samples are tested for N6 by applying validated protocols or sending samples to international reference laboratories for further characterization.

To reduce the risk of spread and transmission, FAO recommends authorities to increase hygiene and biosecurity in live bird markets, to enhance market surveillance and to strengthen traceability to allow efficient and timely traceback of any positive infection to its farm of origin. Poultry batches from affected areas should always be tested prior to importation. The diverse viral gene pool in eastern Asia, established through co-circulation of many different avian influenza viruses, will continue to give rise to novel reassortant viruses, such as H5N6, with unpredictable consequences for animal and human health. Continuous vigilance and monitoring therefore remain needed, along with increased preparedness, especially as the next influenza season is approaching.

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