SCIENTIFIC REPORT OF EFSA

Highly pathogenic avian influenza A subtype H5N8[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Highly pathogenic avian influenza (HPAI) H5N8 outbreaks in poultry farms have been reported in Asia and Europe since January and November 2014, respectively. The entry of HPAI H5N8 into Europe and its subsequent spread within Europe are two separate events with possibly different transmission vectors. Following epidemiological investigations of infected poultry holdings, there is not yet a clear indication of the source of the virus. There are no known direct bird migration routes from Asia to western Europe. It has been hypothesised that long-distance transmission of HPAI viruses could occur as a result of cross-infection between different birds in north Eurasian breeding areas, but this hypothesis needs further investigation. HPAI H5N8 has been detected in wild bird populations in Germany and the Netherlands. Direct contact between wild birds and farmed birds in the affected holdings was unlikely. It is more plausible that indirect introduction of HPAI H5N8 to poultry holdings via humans, vehicles, equipment, fomites, live animals and/or animal-derived products contaminated with virus (for instance in faeces) of infected birds took place. Investigations in the Netherlands suggest separate introductions into four holdings and one between-farm transmission. Assessing biosecurity procedures is recommended with a focus on segregation, cleaning and disinfection, and improving where necessary. Given the apparent low pathogenicity of HPAI H5N8 for several wild bird species, focused strategic and proportionate enhancement of active and passive surveillance of living and dead wild birds in the high risk areas would improve the understanding of the risk of virus transmission to poultry. It might also facilitate the design of targeted measures to reduce the risk of virus transmission between poultry and wild birds. Timely updated analyses on the evolving situation within the European Union are required, as well as assessment of all transmission routes that might transport HPAI viruses from Asia to Europe.

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KEY WORDS

Highly pathogenic avian influenza, H5N8, entry route, wild bird

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SUMMARY

HPAI H5N8 outbreaks in poultry farms have been reported in the Republic of Korea, Japan, China, Germany, United Kingdom and The Netherlands. The first outbreak report in domestic ducks was in the Republic of Korea in January 2014. In Europe, the first affected holding was reported on the 4 November 2014 in the Mecklenburg-Vorpommern region of Germany in a turkey farm. HPAI H5N8 has subsequently been confirmed in a duck farm in East Riding of Yorkshire in the UK and in five poultry farms in the South Holland region of the Netherlands.

This scientific report addresses the mode of entry of HPAI H5N8 into Europe and its potential further spread which are subject to a number of uncertainties. Both initial and subsequent cases have been analysed with the data available to determine if they are all as a result of primary introduction or other factors identified indicating secondary spread. The epidemiological situation and information available until 5 December 2014 was taken into account.

Migratory birds as one possible route of entry into Europe have been suggested based on the role of migratory birds in other avian influenza outbreaks. HPAI H5N8 has been detected in wild birds (common pochard (*Aythya farina*), tundra swan (*Cygnus columbianus*), great egret (*Ardea alba*), bean goose (*Anser fabalis*), Baikal teal (*Anas formosa*), coot (presumably *Fulica atra*), mallard (*Anas platyrhynchos*), white-fronted goose(*Anser albifrons*), common teal (*Anas crecca*) and spot-billed ducks (*Anas poecilorhyncha*) in Asia. There are no known direct bird migration routes from south east Asia to Western Europe. Although the movement of individual birds cannot be excluded, this is highly improbable. It has been hypothesised that long-distance transmission of HPAI viruses could occur as a result of cross-infection between different birds in north Eurasian breeding areas, where wild bird populations from different flyways overlap but this hypothesis needs further investigation. Future analysis of HPAI H5N8 virus sequences from Asia and Europe will be valuable in exploring hypotheses on routes of spread. Furthermore the rates of virus evolution in different populations of both poultry and wild birds is uncertain. However historical data supports higher rates of virus evolution especially upon entry to domestic galliforme species.

Within the European wild bird populations, HPAI H5N8 has been confirmed so far in samples in one common teal in Germany and two faecal samples of Eurasian wigeons (*Anas penelope*) in the Netherlands. The teal was shot in Germany (Rügen island) and showed no indication that its health had been impaired. Field data and preliminary bird experiments indicate that HPAI H5N8 may have a relatively moderate pathogenicity for some wild bird species with limited mortality (<20%). Nevertheless, importantly in infected mallards HPAI H5N8 replicated efficiently and virus shedding was greater than HPAI H5N1 and at levels indicating it could be spread by contact. If the virus is circulating in wild birds, the interface between wild birds and farmed poultry offers a pathway for introduction of the virus into poultry holdings. Spill-over events could also lead to the virus being transmitted from poultry to wild birds. Investigations in the Netherlands using next-generation full genome sequencing with phylogenetic tree analysis suggested separate introductions into four holdings and one between-farm transmission. Close genetic homology among the viral genes of the HPAI H5N8 viruses detected in the United Kingdom, the Netherlands and Germany suggest they all share a common ancestor with the recent Japanese HPAI H5N8 viruses isolated from wild ducks, which is estimated to have occurred in June 2014. However reliable interpretation of the topology of the European and Japanese cluster cannot be made with these similar sequences. Phylogenetic analysis of other viral gene segments and importantly sequences from more viruses will help to resolve these relationships.

The outbreaks in poultry in Europe occurred in indoor facilities; therefore direct contact between wild water birds and the farmed birds in the affected holdings is unlikely. If the virus is circulating in wild waterbird populations contamination of environmental surfaces by faeces and contamination of standing waters through viral shedding might occur. However, data regarding the virus load in the environment due to viral shedding from wild birds are currently lacking. Studies however have demonstrated that at 4°C virus can survive for several weeks in water and may therefore be a clue to

fomite transmission from contaminated environments into housed poultry holdings where biosecurity practices may not be robust.

Given the apparent presence of HPAI H5N8 within some wild bird populations in Europe and the occurrence of HPAI H5N8 infection in several poultry holdings, It is more plausible that indirect introduction of HPAI H5N8 to poultry holdings via humans, vehicles, equipment, fomites, live animals and/or animal-derived products contaminated with virus (for instance in faeces) of infected birds took place . An appropriate biosecurity system is required to prevent virus entry into and virus leaving from a holding since non-avian bridge species such as mice, cats, foxes, rats, dogs and mustelids may act at least as mechanical vectors. The biosecurity systems should also take into account streams of fomites, waste products and water leaving the holding to contain the virus in affected holdings. Detailed epidemiological investigations of the affected European farms and a detailed assessment of all transmission routes that might transport HPAI viruses from south-east Asia to the EU should be continued in order to identify the risk of HPAI introduction into Europe and into European poultry holdings.

Assessing biosecurity procedures at farm and area level with a focus on segregation, cleaning and disinfection, and improving where necessary, is recommended in high risk areas. The probability of introduction and spread of the HPAI H5N8 via contaminated humans, vehicles, equipment, fomites, live animals and/or animal-derived products is dependent on several factors like the prevalence of the virus, stability of the virus under the conditions prevailing at the time and characteristics of the fomite (e.g. water content of the material). Knowledge of the prevalence and pathogenesis of HPAI H5N8 infection in wild bird populations is required in order to better understand the risk of transmission to poultry, which is important in the design of risk management strategies. Given the apparent low pathogenicity of HPAI H5N8 for several wild bird species, focussed strategic and proportionate enhancement of active (targeted) and passive (scanning) surveillance of both living and dead wild birds in the high risk areas is recommended.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Highly pathogenic avian influenza (HPAI) H5N8 viruses have been reported in Asia since 2010 and more recently in January 2014 in the Republic of Korea affecting, domestic poultry and wild birds; in April 2014 in Japan affecting domestic poultry and in October 2014 in China, in domestic poultry. Different species of wild birds have been found infected with HPAI H5N8 in those countries; most recently in mid-October the virus was detected in a Tundra swan in Japan.

On 6 November 2014, an outbreak of HPAI H5N8 was confirmed in one of five sheds at a turkey holding in the north-east of Germany. The virus was identified as similar to the one previously identified in South Korea. A second outbreak was notified on 15 November in the Netherlands. It concerned a holding with laying hens kept indoor and located north east of Rotterdam. The virus was confirmed to be HPAI H5N8. One day later, the United Kingdom notified also an outbreak of HPAI virus of the H5 subtype at an indoor duck breeding holding in the East Riding of Yorkshire. Also in this case, the virus was confirmed to be HPAI H5N8.

The affected Member States immediately applied the measures foreseen by Council Directive 2005/94/EC in order to control the spread of the virus and the European Commission adopted swiftly certain protective measures in relation to these HPAI H5N8 outbreak and zoning measures were put immediately in place in the well-defined disease area (affected zone) which guarantee no further spreading of the virus to other parts of the European Union and to trading partners.

These three H5N8 outbreaks in Europe have been reported in a short time period, and were located at distant locations and affected three different poultry species and production systems. Epidemiological outbreak investigations are still on-going in the affected Member States in particular to identify the possible source of virus. The fact that the recent outbreaks in Germany and The Netherlands have occurred in proximity of humid areas with high wild bird densities and the lack of evidence of any other possible epidemiological link between them point towards wild migratory birds as a possible source of virus introduction. Apparently certain species of wild ducks and swans might be carrying the virus without showing signs of disease. EU Member States are evaluating their wild bird surveillance data and are enhancing monitoring.

The European Reference Laboratory for avian influenza (EURL) collates data provided by the Member States to the Commission on wild bird and poultry surveillance activities, other data provided by epidemiological reports of the affected Member States and also laboratory data from on-going investigations. EFSA could contribute to some of the activities of the EURL by giving scientific support in the identification of the epidemiological data required to better understand the epidemiological situation and by facilitating the interaction between the EURL and the affected Member States via the EFSA network on animal health and welfare. The use of the EFSA Data Collection Framework (DCF) as a data exchange portal will be a valuable asset to collect additional information from Member States in a structured manner.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

1. Epidemiological analysis of the current situation regarding HPAI subtype H5N8 in Europe in order to assess possible entry routes and in particular the role played by wild birds.
2. Review of the epidemiological situation of HP AI subtype H5N8 in the world.

EVALUATION

1.   Introduction

Since January 2014, HPAI H5N8 has caused outbreaks in poultry in the Republic of Korea, Japan, and China (see chapter 2). This virus is primarily of concern to poultry production; to date, there have been no reports of human cases. The risk for zoonotic transmission to the general public in the EU/EEA countries is considered extremely low (ECDC, 2014).

Although HPAI H5N8 also was detected in free-living wild birds in the Republic of Korea (Jeong et al., 2014), their role in the epidemiology of this virus is not clear. In November 2014, HPAI H5N8 has again been reported in both poultry and wild birds, not only in Japan, but also in Germany, the Netherlands, and the U.K. This scientific report gives an overview of the confirmed HPAI H5N8 cases in poultry and wild birds, describes the genetic characteristics of the currently circulating HPAI H5N8 viruses and described possible entry routes of HPAI H5N8 into Europe. The epidemiological situation and information available until 5 December 2014 was taken into account.

2.   Description of the reported HPAI H5N8 outbreaks

Republic of Korea: In mid-January 2014, an outbreak of H5N8 was reported, causing disease in breeding ducks and chickens (Lee et al., 2014) (Figure 1 and Figure 2). Domestic outbreaks were also associated with die-offs of Baikal teal (*Anas 6arina6)* and bean geese (*Anser fabalis*) near the first reported poultry cases in Jeonbuk province (Jeong et al., 2014). The authors reported that Donglim Reservoir is considered as one of the most important sites for migratory Baikal teal, which overwinter in the Republic of Korea, Japan and China (Kear, 2005). The detection of poultry and wild birds infected with HPAI H5N8 viruses having a high level of nucleotide identity at very close distance to each other and in the same period suggested that these viruses share a common ancestor (Jeong et al., 2014; Kim et al., 2014; Lee et al., 2014).

In the last week of January 2014, the outbreak spread to north and south in the migratory bird habitats of western Korea—referred to as phase II of the Korean outbreaks—and HPAI H5N8 viruses were obtained from the carcasses of Baikal teal, mallard (*Anas platyrhynchos*), bean goose and coot (presumably *Fulica atra*) (Figure 2 and Figure 3). Chicken and poultry holdings in six provinces were confirmed to be infected with HPAI H5N8. In each province, detection of infection in wild birds was geographically close to poultry holdings (Jeong et al., 2014).

The outbreak continued to spread further. Ku and colleagues (2014) isolated H5N8 from waterfowl in the Pungse River in Chungnam Province in February 2014. Further 161 poultry holdings and 20 wild birds were confirmed as HPAI H5N8 positive by 8 May 2014 (phase III) (Figure 3) (Jeong et al., 2014).

Additional cases in domestic ducks and geese were reported in June, July and September 2014 (Figure 3).

Japan: An outbreak in a chicken holding was reported in March 2014 (Figure 1 and Figure 2). No further outbreaks were reported until detection of HPAI H5N8 in a tundra swan (*Cygnus columbianus*) on 3 November 2014, a common pochard (*Aythya 6arina*) on 13 November and in an unidentified wild duck on 18 November 2014. Japan shares interconnected wild bird migratory routes with Korea as they both lie within the major East-Asian Australasian Flyway which connects waterbird breeding areas in arctic Russia with non-breeding areas in Australasia (McCure, 1998; Miyabayashi, Y. and Mundkur, T. 1999; Straw et al. 2006).
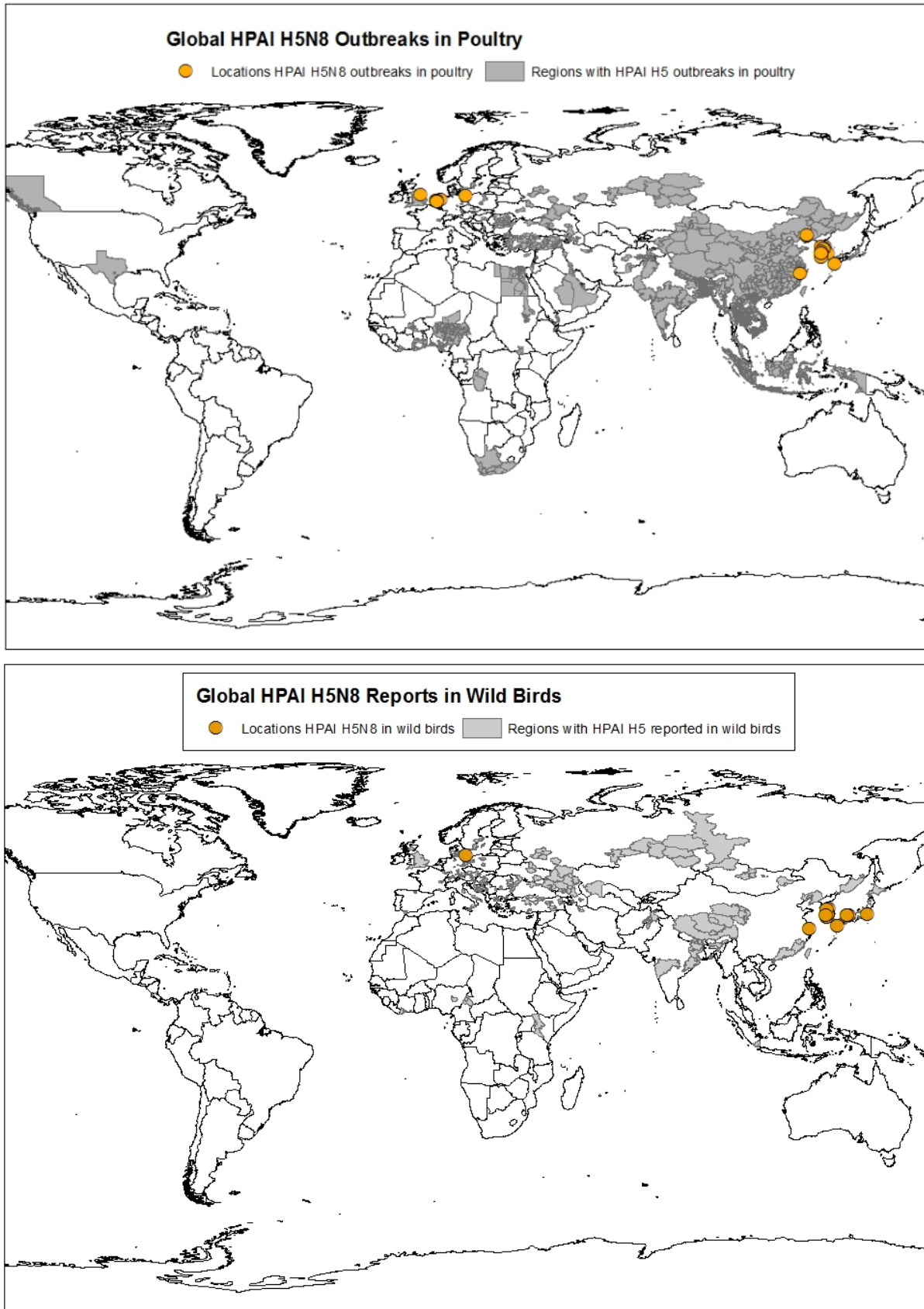
Figure 1: Geographical distribution of regions that experienced past HPAI H5 outbreaks in both poultry and wild birds (as grey shading) with HPAI H5N8 outbreak locations (as orange points) (Empress data extracted 28 November 2014 for Avian Influenza covering the period 2003 – 2014)
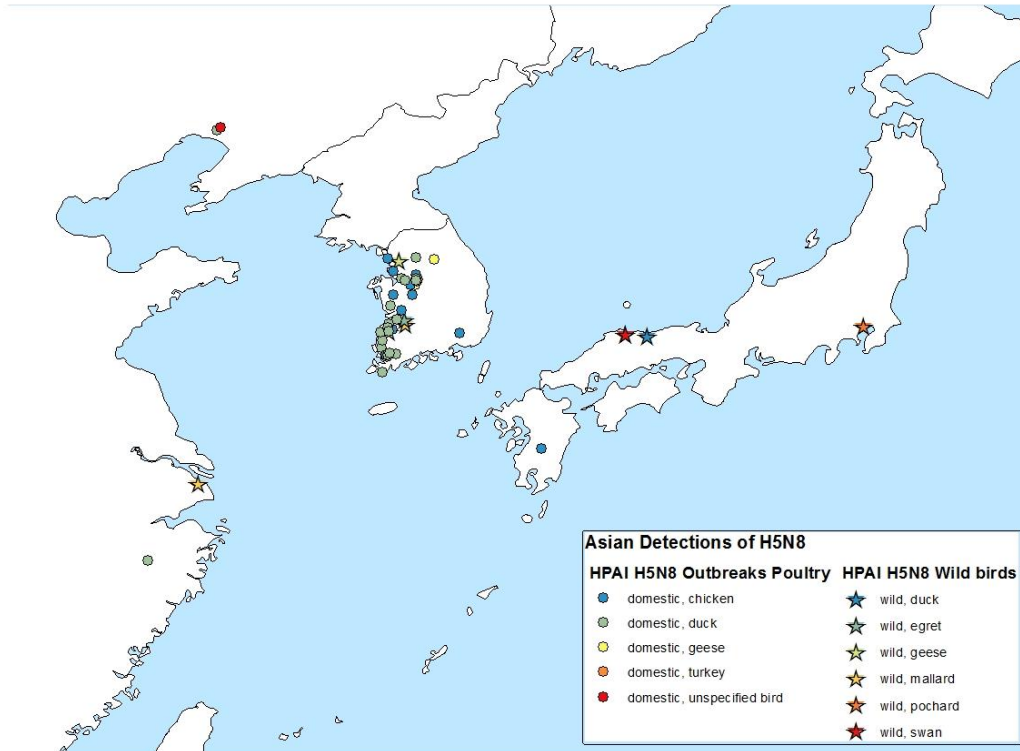
Figure 2: Locations and affected species of confirmed cases of HPAI H5N8 in Republic of Korea, Japan and China (Empress data extracted 28 November 2014 for Avian Influenza 2003 – 2014)
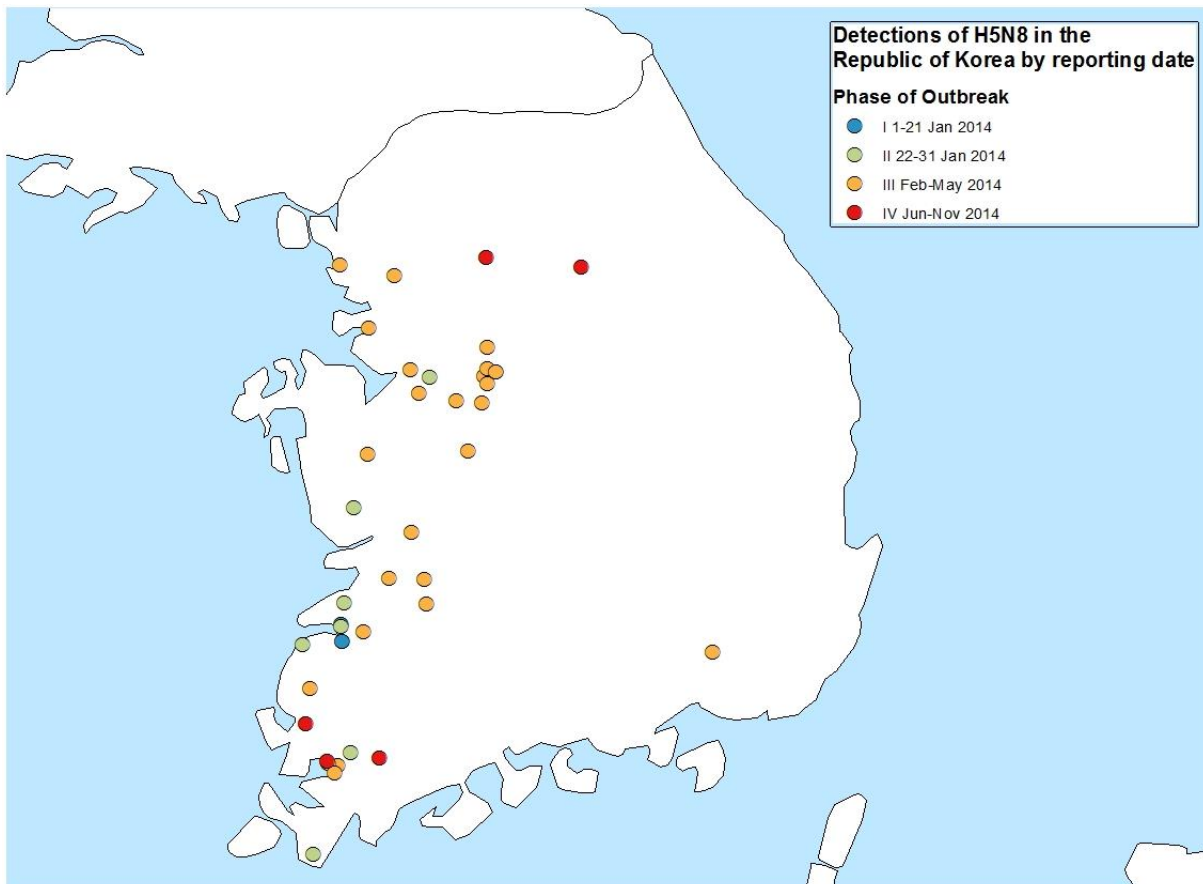
Figure 3: Detections of HPAI H5N8 in the Republic of Korea by phase of outbreak

China: The progenitor strains of the current HPAI H5N8 were detected in wild birds in China as far back as in 2010 (see chapter 3). In May 2014, HPAI H5N8 was detected in domestic ducks in Zhejiang Sheng and in September 2014 HPAI H5N8 was detected in poultry in Liaoning Sheng which was reported to be in the Liao river wetlands (Figure 1). Kim and colleauges (2014) did not consider it surprising that HPAI H5N8 circulated in China, the Republic of Korea and Japan since they share interconnected wild bird migratory routes. This statement might be based on a similar observation with HPAI H5N1 outbreaks in the region (Sonnberg et al., 2013) as a detailed analysis was not provided. It is important to note that the outbreak in May is situated south of Korea and Japan while bird migration in spring leads from south to north. There may also have been vectors other than wild birds involved.

Europe: As at 5 December 2014, HPAI H5N8 has been confirmed in seven poultry holdings in Europe (Table 1, Figure 1 and Figure 4). The first affected holding was reported on the 4 November 2014 in the Mecklenburg-Vorpommern region of Germany in a turkey farm. Subsequently HPAI H5N8 was confirmed in one duck breeding facility in the East Riding of Yorkshire in the United Kingdom (UK) and in four chicken holdings and one duck holding in the Netherlands in the Utrecht, Zuid-Holland and Overijssel regions. In all seven farms, the poultry are kept in indoor housing.

Table 1: Characteristics of affected holdings in Europe

Country	Region, city	Species	Flock size	Month reported	Day reported	Confirmed subtype	Indoor Housing
DE	Mecklenburg-Vorpommern	Turkey (fattening)	30939	11	4	H5N8	Y
NL	Utrecht, Hekendorp	Chicken (broilers, layers)	150000	11	14	H5N8	Y
NL	Zuid-Holland, Ten Aar	Chicken (layers)	43000	11	19	H5N8	Y
NL	Overijsel, Kamperveen	Chicken (breeding)	10000	11	21	H5N8	Y
NL	Overijsel, Kamperveen	Duck	15000	11	22	H5N8	Y
GB	East Riding Of Yorkshire, Nafferton	Duck (breeding)	6178	11	1	H5N8	Y
NL	Zuid-Holland, Zoeterwoude	Chicken (layers)	28000	11	29	H5N8	Y
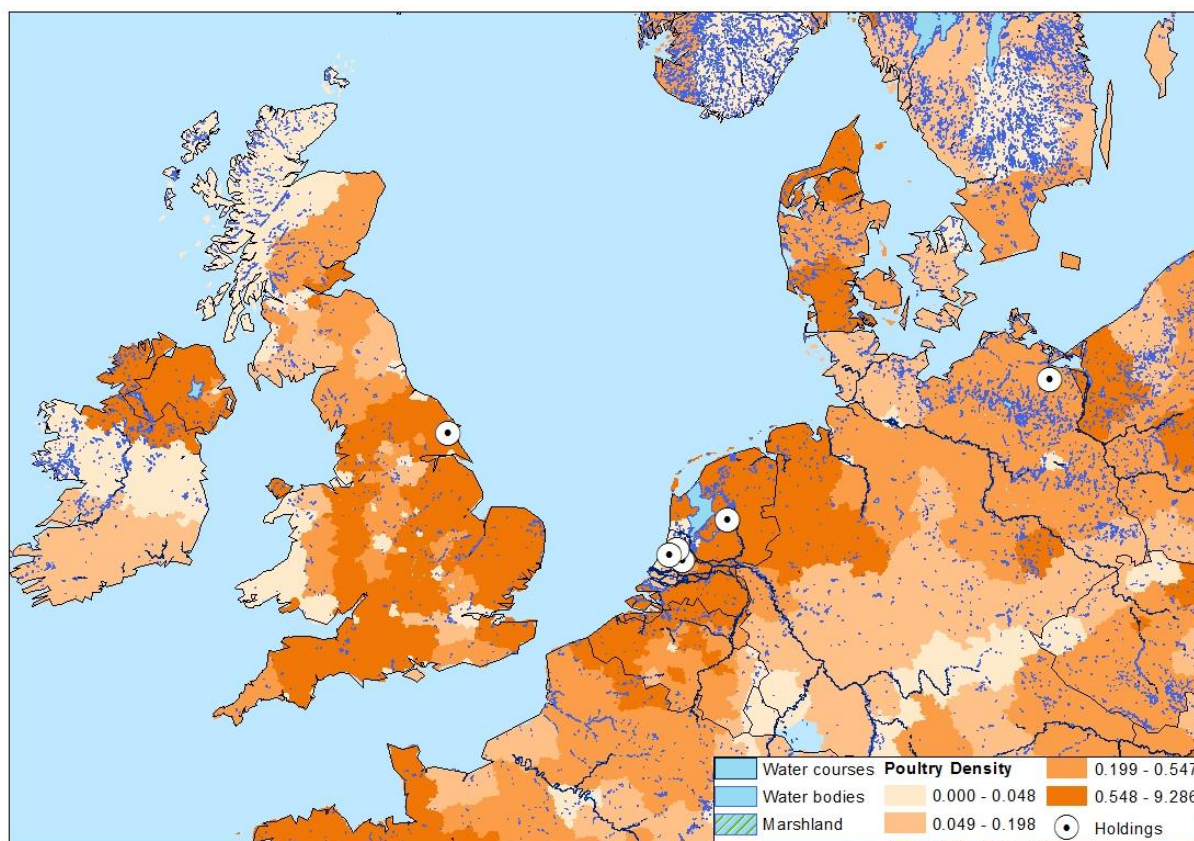
Figure 4: Location of holdings with laboratory confirmed HPAI H5N8 (Corine 2000/2006 water courses, water bodies and marshland (European Environment Agency), Poultry density 1000 heads poultry / square km at NUTS3/NUTS2 (Eurostat holding structures survey, 2010))

The Eurostat Farm Survey from 2010 records the number of poultry holdings and number of heads of poultry at NUTS3/2 level. Poultry densities in Europe range from zero to 9,286 individuals per km^2 (median value of 196 individuals per km^2). The affected regions in the Netherlands and the United Kingdom are in the upper quartile for poultry density in Europe. The affected farms in the Netherlands were not located in regions with the highest poultry density of the country.

Table 2: Number of poultry holdings and heads of poultry in the affected regions (Eurostat holding structures survey 2010)

Country	Region	Poultry (1000 heads)	Number of Poultry Holdings	Poultry Heads / km^2	Holdings/ km^2
DE	Mecklenburg-Vorpommern	9160	740	397	0.032
NL	Utrecht	2350	120	1615	0.082
NL	Noord-Overijssel	4170	110	2790	0.074
NL	Oost-Zuid-Holland	280	20	590	0.042
UK	East Riding of Yorkshire	2740	260	1144	0.109

The following features and observations have been reported for the outbreaks:[4]

DE:
- Suspicion was based on clinical signs and gross increase of mortality
- Fattening turkeys in 6 houses of the holding since 21/07-29/09/2014 (purchase of day old turkeys from a hatchery in Germany)
- Pathology turkeys: herds of pancreatic necrosis associated with fibrinous exudates, necrosis of ileo-caecal tonsils, discrete petechial haemorrhages were present in peri- and subepicardial locations; heavily injected subserosal mesenteric vessels dominated the situs.
- Large flocks of wild birds observed close to the holding
- The turkey holding in Germany is in the Mecklenburg-Vorpommern region where HPAI H5N1 was reported in the spring of 2006 in wild swans, geese, ducks and other avian species (Figure 1)
- In Mecklenburg-Vorpommern, there are 397 heads of poultry per square kilometre respectively (Figure 4, Table 2)

NL:
- Mortality up to 7% in affected poultry house (related to the first outbreak in Utrecht)
- Confirmed positive results in 1/6 poultry houses in the affected holding (related to the first outbreak in Utrecht)
- In the affected regions of Zuid-Holland, Overijssel and Utrecht, 590, 2790 and 120 heads of poultry per square kilometre respectively (Figure 4, Table 2)
- An increased mortality was observed in the affected holdings without a drop in feed and water intake
- Remarkably all chicken farmers reported that mortality was the most striking clinical sign. None of them observed reduced food and water intake and/or reduced egg production in the day(s) before mortality increased.
- It is noteworthy that the affected holdings were in areas that have more wetlands and concentrations of migratory waterbirds than the two most poultry-dense areas in the Netherlands, Gelderse Vallei and Noord-Brabant/Limburg.
- Duck holding: mortality was 0.03, 0.06, 0.17, 0.07, 0.28% on the days preceding the culling; only signs listless and increased mortality.

UK:
- Clinical signs were reduced egg production and small increase in mortality on a duck breeding holding
- Pathology: not classical HPAI, concurrent disease complicated the clinical picture; severe air sacculitis, haemorrhagic eggs and follicles, peritonitis, coelomitis, splenomegaly, necrosis in pancreas
- The holding has 6 sheds – 3 were empty having been depopulated on the 5/6 November 2014. Carcasses of these three sheds have been traced.
- A field ornithological evaluation of the site and surrounding areas found it was not attractive to wild birds with no large numbers of waterfowl (or 'bridge species' either at the site or nearby;
- The sheds were protected with maintained netting to prevent incursions by wild birds, and there was no evidence to suggest significant use of the site was made by wild birds (*e.g.* sources of food to attract gulls *Larus* spp.).
- In East Riding of Yorkshire, there are 1144 heads of poultry per square kilometre respectively (Figure 4, Table 2)

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[4] Based on presentations of PAFF meeting (20 November 2014; http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/presentations_en.htm#20141120 last accessed 24 November 2014) and on information provided to EFSA by Member State representatives

3.   Characteristics of the circulating HPAI H5N8 viruses

In Asia, HPAI H5N8 viruses have been found since 2010 (Dk/Jiangsu/k1203), but none of those previous strains resembled the genetic components of this 2014 viral pathogen nor did they cause local outbreaks (Wu et al., 2014) (Figure 5). A reassortant between HPAI H5N8-like and A(H11N9)-like strains has been isolated in China in December 2013 (Dk/Zhejiang/W24), which are genotypically very similar to H5N8 virus circulating in the Republic of Korea since the beginning of 2014 (BDk/Gochang1). In these outbreaks, a genetically distinct group of reassortant HPAI H5N8 viruses containing A(H4N2) sequences has been detected (e.g. BTI/Donglim3) (Kim et al., 2014; Lee et al., 2014; Wu et al., 2014) (Figure 5). It is unclear which HPAI H5N8 virus lineage (the 2010 Jiangsu-like or 2013 Zhejiang-like strain) participated in this reassortment. This group of HPAI H5N8 viruses seems to be dominant in the 2014 outbreaks in south east Asia and Europe. There are no indications of the H9N2-like segments that were found in human-infecting H7N9 and H10N8 avian viruses in China (Kim et al., 2014).

Current circulating HPAI H5N8 viruses, together with A(H5N5) and A(H5N6) viruses, belong to clade 2.3.4.6 of the HA gene (Lee et al., 2014; Jeong et al., 2014). They have more than 99.5% nucleotide identity in the HA gene with viruses circulating in Korea and less than 99.2% compared to viruses circulating in China or Japan. The currently circulating HPAI H5N8 viruses are still essentially avian in their phenotype and lack some of the key changes increasing affinity for replication in humans. In addition, sensitivity to antiviral drugs is predicted (Personal communication, Ian Brow, EURL, 05 December 2014).

Initial Maximum Likelihood (ML) phylogenetic analysis of the HA gene revealed that the HPAI H5N8 viruses circulating in the three affected Member States have 99.7% or higher nucleotide identity amongst each other and their HA cleavage site motive RNSPLRE**RRRKR***GLF is conserved. Sequence comparisons based on 1608 nucleotides of the HA gene between the UK and Germany identify 99.8% similarity. Further phylogenetic analysis was implemented using Bayesian Markov chain Monte Carlo simulation in the BEAST package v.1.7 for the viruses from the UK, the first case in the Netherlands and that from Germany with others publicly available. The maximum clade credibility tree (Figure 6) had a similar topology to that observed for the ML tree. The time to the most recent common ancestor for the European and Japanese cluster is 5 months (June 2014), 95% highest posterior density (HPD) range 2.7-7.7 months. This European and Japanese cluster shares homology in the HA gene with viruses detected in Korea in early 2014. The ancestor of the European, Japanese and Korean viruses occurred 13 months ago (October 2013), 95% HPD 11-15.5 months. High sequence similarity precludes reliable estimation of when European and Japanese viruses diverged (personal communication, Ian Brow, EURL, 05 December 2014).

Phylogenetic analyses of all available European and Asian HPAI H5N8 isolates using Bayesian Markov Chain Monte Carlo analyses suggests that four of the five outbreaks of HPAI H5N8 virus in the Netherlands were caused by separate introduction and not by farm-to-farm spread. In addition the analysis suggests a between-farm transmission between the third and fourth outbreak (both in Kamperveen), although it cannot be entirely excluded that both outbreaks resulted from two separate introductions from the same source. The first four Dutch isolates had maximal 20 nucleotide substitutions across the whole genome that should have been generated during circulation in poultry during 9 days if it is assumed that between farm spread had continued the spread after the index case (personal communication, Ruth Bouwstra, CVI Lelystad, 05 December 2014).

During the HPAI H7N7 outbreak in the Netherlands maximal 25 substitutions were generated in 256 outbreaks in 9 weeks and in Italy 66 substitutions in 9 months (Jonges et al., 2014). Remarkably, H5N8 viruses isolated from non-specified species of *Anatidae* in Chiba in Japan in November 2014 most likely derived from the same precursor as viruses isolated in Europe. These results could be consistent with a hypothesis that the precursor virus was present in Siberia on the breeding grounds where migratory birds from the East Atlantic Flyway and East Asian Australasia Flyways may have mingled during the breeding season in 2014. The fact that European viruses diverted from the same

Figure 5: Putative generation of the novel HPAI H5N8 viruses that caused domestic outbreaks in Asia and Europe in 2014 (Kim et al., 2014)
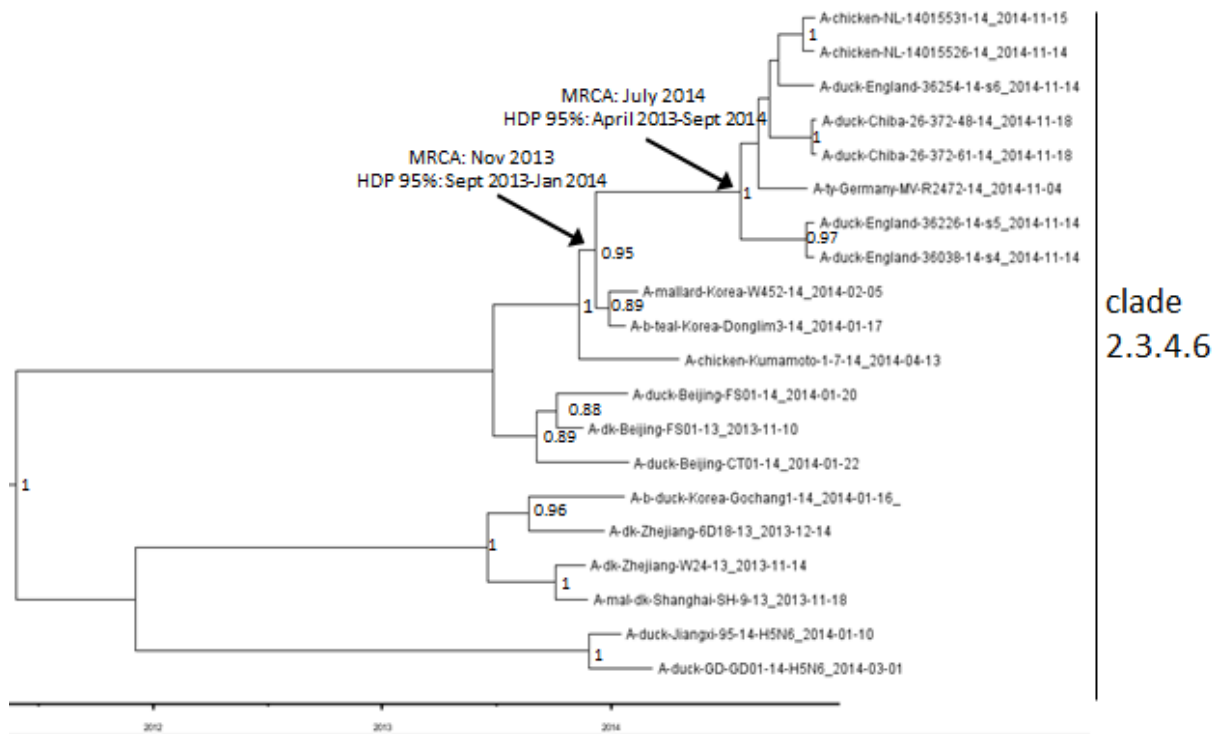
Figure 6: Maximum Clade Credibility tree of 20 H5 sequences derived from the haemagglutinin gene of avian influenza viruses belonging to clade 2.3.4.6 (1608 nucleotides). The sampling dates and locations are included on the tip labels. The node labels are significant posterior probabilities (>0.75). The dates for the most recent common ancestor (MRCA) of the currently circulating European and Japanese viruses are indicated at the relevant nodes with 95% highest posterior density (HPD) levels.

ancestor as two Japanese viruses suggests that there has been little opportunity for the accumulation of mutations during movement between Asia and Europe. If many bird species were involved, greater number of nucleotide differences between European and Japanese viruses may be expected (Personal communication, Ruth Bouwstra, CVI Lelystad, 03 December 2014; Personal communication, Ian Brow, EURL, 05 December 2014).

4.   Assessment of possible entry routes for H5N8 into Europe

Introduction and spread of HPAI into poultry farms of a country has been linked in the past to movement of infected or contaminated persons, vehicles, equipment, fomites, live animals and/or animal-derived products (EFSA, 2008). Only the westward spread of HPAI H5N1 from Asia to Europe indicated that migrating wild birds could play a possible additional role (Liu et al., 2005; Olsen et al., 2006; Gauthier-Clerc et al., 2007; EFSA, 2008; Si et al., 2009; Gilbert and Pfeiffer, 2012). A descriptive assessment of these possible entry routes for HPAI H5N8 is presented below. It is important to realize that entry of the infection into Europe (single or multiple events) and subsequent further spread in Europe are two separate events which might involve different transmission routes. Given the specific emphasis on wild birds in the EFSA mandate provided by the European Commission, the possible role of wild birds is described first.

4.1.   Possible long-distance spread of HPAI H5N8 to Europe via migratory birds

Most wild birds infected with HPAI H5N8 were reported in January-February 2014 from in several provinces in the Republic of Korea. The detection of HPAI H5N8 in wild birds was associated with H5N8 outbreaks in poultry (see chapter 1). The virus was detected in apparently healthy birds as well as in carcasses of dead birds and H5N8-specific antibodies were detected in some wild bird species. This provides an indication that H5N8 was widespread in some wild bird species, and had variable clinical effect: subclinical infection in some wild birds, fatal infection in others.

Since November 2014, HPAI H5N8 has been reported in wild birds in four places in Japan, one in Germany and one in the Netherlands. A detailed analysis of the Japanese cases would be informative, because, just like in Europe, they are being reported concurrently with post-breeding migration of wild birds.

The probability of HPAI H5N8 being transported via migratory birds into Europe is highly dependent on the wild bird species involved being infected with the virus, the number of infected birds moving towards Europe and the pathogenicity of the virus strain in the species concerned.

Wild birds species that have been confirmed infected with HPAI H5N8 in south east Asia are the common pochard (*Aythya ferina*), tundra swan (*Cygnus columbianus*), great egret (*Ardea alba*), bean goose (*Anser fabalis*), Baikal teal (*Anas formosa*), coot (presumably *Fulica atra*), mallard (*Anas platyrhynchos*), white-fronted goose (*Anser albifrons*), common teal (*Anas crecca*), spot-billed ducks (*Anas poecilorhyncha*) and unidentified wild ducks and geese (see Table 4, Appendix A). However, it cannot be excluded that other wild bird species have also been infected with HPAI H5N8.

Regarding the pathogenicity of HPAI H5N8 for wild birds, information from field and laboratory suggests the level of clinical disease from HPAI H5N8 infection in wild birds ranges from no clinical disease to death. HPAI H5N8 infection was associated with mortality of more than hundred Baikal teal, in 10 of which HPAI H5N8 was detected; it was found in smaller numbers of dead birds of multiple other species (bean goose, mallard, coot, white-fronted goose, tundra swan), all in South Korea (Jeong et al., 2014). However, it was also detected in swabs of apparently healthy birds: common teal, spot-billed duck, and mallard in South Korea; common teal in Germany and Eurasian wigeon in the Netherlands. Also, anti-H5 antibodies, suggesting recovery from H5N8 infection, were found in multiple species in the Republic of Korea: Baikal teal (16/30, 53%), Eurasian wigeon (6/12, 50%), spot-billed duck (26/146, 18%), mallard (54/379, 14%), and common teal (5/50, 10%) (Jeong et al., 2014). Experimental infection of five wild mallards resulted in either no or mild clinical signs of

disease (Kang 2014). Experimental infection of two Baikal teal did not result in clinical signs. One bird died at 3 dpi, but cause of death was not determined (Kang et al., 2014).

The migration of wild birds is complex with a wide variety of migration strategies between, and even within, different bird species. Migration routes can vary (1) by species (and by population within species) and the extent of migratory path can vary, both by total length of flight-path and by number and duration of stops along flight-path ('hop, skip and jump' strategies), (2) by age of individual; (3) by sex of individual; (4) by individual; (5) by season; and (6) due to weather (EFSA, 2006). Therefore, maps of migratory pathways have to be interpreted with caution and are typically broad generalisations.

Nearly all migration systems link higher latitudes in the breeding season with lower latitudes in the non-breeding season. It is therefore wrong to assume that there are track directions within the generalised mapped 'flyways' that run perpendicular to the general north-south orientation of most flyways. Generalised illustrations of migratory flyways are attractive to report as they look very simple but hide much ecological complexity and can easily be misinterpreted by those unfamiliar with the details.

Information on bird movements comes from two sources.

a)   Recoveries of conventionally ringed birds. This information links locations where the bird was marked with where it was recovered (possibly many years later) (Figure 8). These data depend heavily on the distribution of ringers, of people recovering the marked birds, of reporting probabilities and, for waterbirds especially, on the distribution of hunting activities.

b)   More detailed information on the movement tracks of individual birds comes from the use of various remote sensing technologies. These give detailed movement tracks for individually marked birds (e.g. Figure 7).

Data recorded by FAO-USGS Avian Influenza projects (Figure 7) confirm that migratory routes of species studied with positional recorders lead along north-south flyways, with a more prominent north-east to south-west component in some wild bird populations wintering in Europe. Indeed, there are no known direct bird migration routes from eastern Asia to western Europe, although the movement of individual birds cannot be completely excluded although this is highly improbable. In 2014, there were also no unusual weather conditions observed (e.g. extremes of temperature or precipitation) which could have induced unexpected wild bird migration to Europe from east Asia.
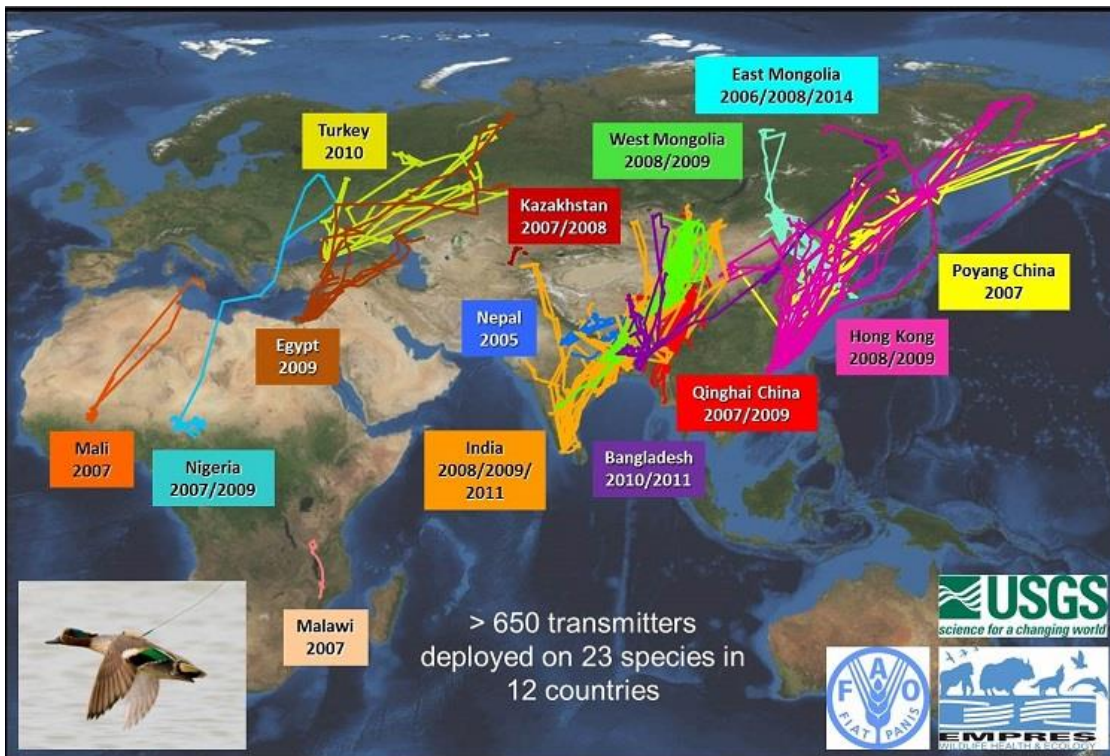
Figure 7: FAO-USGS Avian Influenza Projects[5] (http://www.werc.usgs.gov/ResearchTopicPage.aspx?id=17) ©FAO

Instead of direct wild bird migration from south east Asia to Europe, it has been hypothesised that long-distance transmission of HPAI viruses might occur as a result of cross-infection between different birds where flyways overlap (typically on northern breeding areas) (e.g. Si et al., 2009; Iverson et al., 2011). This transmission could only take place if wild birds from two different flyways have direct contact with each other, indirect contact via an intermediate host, or if the virus retained its infectivity in the applicable environmental conditions and indirectly infected another wild bird. Breeding grounds in Siberia could be a possible overlap area between migratory birds coming from Europe and from south-east Asia (Gilbert et al., 2006; Shestopalov et al., 2006; Ilyicheva et al., 2013).

As mentioned before, sequence analysis suggests that HPAI H5N8 viruses isolated from non-specified species of *Anatidae* in Chiba in Japan in November 2014 are most likely derived from the same precursor as viruses isolated in Europe (Figure 6). These results could be consistent with a hypothesis that the precursor virus was present in Siberia on the breeding grounds where migratory birds from the East Atlantic Flyway and East Asian-Australasia Flyways may have mingled during the breeding season in 2014. The fact that European viruses may be derived from the same ancestor as two Japanese viruses suggests that there has been little opportunity for the accumulation of mutations during movement between Asia and Europe. Data on both viral sequence analysis and wild bird migration should be taken into account when assessing the role of wild birds in the introduction of HPAI H5N8 from south-east Asia into Europe. Information from conventional bird ringing summarised by the Migration Mapping Tool (http://blx1.bto.org/ai-eu/) can be used to indicate broad migration patterns for different wild bird species from Europe into central Asia. For example, Figure 8 show recovery data for the European wigeon (confirmed infected with H5N8 in the Netherlands).

There are no synthesised data available on timing and quantities of wild birds migrating from south east Asia to central or north Asia, hampering estimations of possible transmission of HPAI viruses between migratory routes a) from south-east Asia to central/north Asia, and then b) from that region to Europe. It can be assumed that at least one full migration cycle would be required to allow transmission of HPAI between adjacent flyways in order to bring the virus into Europe. Furthermore,

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[5] FAO granted approval to present the figure in this scientific report (04 December 2014)

there is also high uncertainty due to the lack of data on inter-species transmission dynamics of HPAI viruses, in particular for H5N8.
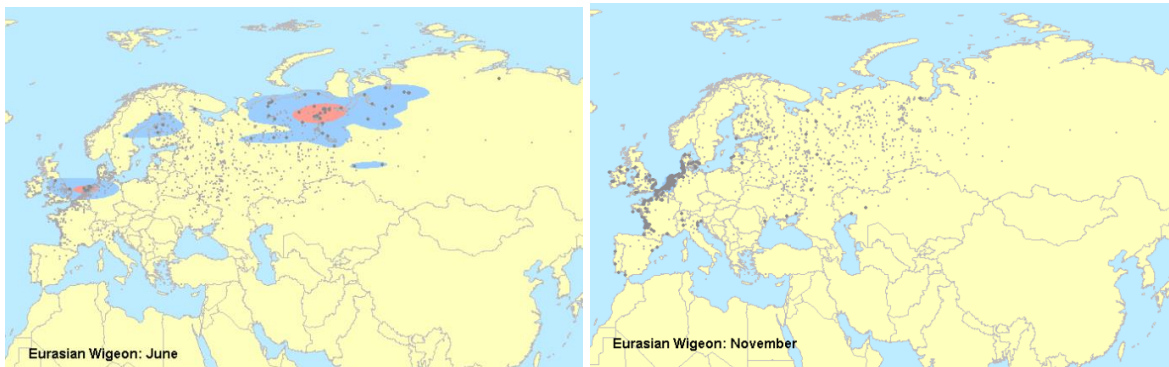

Figure 8: Main migratory movement pathways for Eurasion wigeon based on ringing recoveries in June and November (source: Migration Mapping Tool) ©MMT

The number of HPAI H5N8 infected migratory birds arriving in Europe (if at all) is dependent on the virus prevalence in the region of origin and stop over places, the probability of virus transfer between birds from different migratory routes, the possibility that infected birds to survive migration, and the likelihood that birds are shedding virus (i.e. are infectious) when they arrive in Europe. It is not possible to generate precise estimations due to the lack of data on all the parameters. More knowledge on all these parameters as well as the pathogenicity of HPAI H5N8 in different wild bird species is required to assess the probability of migratory birds transporting the virus from south east Asia to EuropeIt cannot be concluded that the virus is absent in central and north Eurasia since there is a lack of information on the sensitivity of the HPAI surveillance performed in these regions.

From the above, it can be concluded that HPAI H5N8 can infect certain wild bird species without evoking clear disease. Entry of the virus through wild birds into Europe from south-east Asia seems highly improbable, because of the lack of migratory routes and the genetic similarity of Asian and European viral genotypes. Nevertheless, entry through migratory birds breeding in Siberia, that possibly were in contact with birds wintering in south-east Asia, or that have been exposed to a common source, could be possible although this hypothesis needs further investigation.

Given the finding of H5N8 in wild birds in Europe, its presence in at least some wild birds in Europe is now a fact, regardless of the original route of transmission to Europe. Consequently, maintenance of the virus in wild bird populations in Europe is now of concern. Should that happen, possible spill-over from wild birds to poultry might be a risk for a longer period in affected regions.

4.2. Possible local transmission of HPAI H5N8 from wild birds to poultry in European holdings

Lists of European bird species with higher probability to be exposed to HPAI H5N1 viruses were generated in 2005 (EFSA, 2006; Veen et al., 2007). Around 30 species were suggested as possible bridge species that might act as a link between wild birds and poultry. These comprise both migratory and non-migratory bird species that are more widespread and less dependent of wetland habitats, including species that come into more regular contact with poultry and humans. They are seen as having the potential to bridge the gap between avian influenza infected waterbirds and poultry. However, there is high uncertainty on the role of bridge species in the spread of avian influenza viruses due to the lack of data on inter-species transmission dynamics as well as on the consequences of infection of European wild birds. In addition, the species possibly involved are likely to vary greatly between different European regions (EFSA, 2006).

Since 2003 EU Member States have been implementing surveillance programmes for avian influenza in particular aiming to detect infections with high pathogenicity H5N1 HPAI. The spread and multiple

detections of H5N1 HPAI of the Asian lineage have indicated the utility of surveillance and early detection systems, both in poultry and wild birds, until 2006 (Hesterberg et al., 2009).

Council Directive 2005/94/EC on Community measures for the control of avian influenza requires that Member States carry out surveillance programmes for avian influenza according to harmonised guidelines which have last been laid down in Commission Decision 2010/367/EU. The most recent European Commission guidelines on surveillance for HPAI H5N1 in wild birds includes a list of "Target Species" that incorporates knowledge of the number of detections of HPAI H5N1 in the EU surveillance programme and findings on the epidemiology of this virus in wild birds. One of the objectives of EU wild bird AI surveillance, according to Commission Decision 2010/367/EU, is the timely detection of HPAI of the subtype H5N1 in wild birds in order to protect poultry in poultry holdings and safeguard veterinary public health.

The objectives of the surveillance programme for avian influenza in poultry (as described in Commission Decision 2010/367/EU) are to inform the competent authority of circulating avian influenza virus with a view to controlling the disease in accordance with Directive 2005/94/EC by the annual detection through active surveillance for: (a) low pathogenicity avian influenza (LPAI) of subtypes H5 and H7 in gallinaceous birds (chickens, turkeys, guinea fowl, pheasants, partridges and quails) and ratites thereby complementing other existing early detection systems. (b) LPAI of subtypes H5 and H7 and highly pathogenic avian influenza (HPAI) in domestic waterfowl (ducks, geese and mallards for re-stocking supplies of game).

If HPAI H5N8 were to be present in wild bird populations in the EU and cause significant morbidity or mortality as occurs with HPAI H5N1, then wild bird passive (or scanning) surveillance activities of MS would have utility for the detection of this virus if undertaken at a sufficient scale. In contrast however, if HPAI H5N8 was carried only asymptomatically in wild birds, active (or targeted) surveillance would be a more appropriate method. Variability in mortality due to infection with HPAI H5N8 within and among species may result in both active and passive surveillance having some utility.

Since the implementation of Commission Decision 2010/367/EU there has been no mandatory requirement for Member States to carry out active (or targeted) surveillance programmes in wild birds (sampling healthy live or hunted birds). Active surveillance is expected to have utility for detection of avian influenzas that do not cause significant morbidity or mortality in wild bird populations.

The number of birds sampled by passive surveillance has decreased since its peak in 2006 (Figure 9) and the limited number of birds currently tested may result in a reduced sensitivity of the current surveillance system. It is possible to get valuable and useful results from proportionately very small samples of a population. The utility of this sampling really depends upon prevalence and the spatial distribution of sampling in relation to natural heterogeneity in the spatial distribution of infected individuals. Ten populations of nine target bird species were not sampled in 2013. However, it should be noted that data on active surveillance are not submitted by certain Member States and there are large variations in sampling densities amongst regions (Figure 10). It is expected that current HPAI H5N8 outbreaks in the EU may lead to increased surveillance activities in Q4 of 2014 compared to Q4 in 2013. Active (or targeted) surveillance of live wild birds, freshly-expelled faeces and/or feathers in high risk areas might be useful for HPAI H5N8 in species which the virus have low virulence several wild bird species.

Inoculation experiments showed that the virus systemically replicated and was lethal to chickens (Zhao et al., 2013; Kim et al., 2014 EMI; Wu et al., 2014). This virus efficiently transmitted in domestic ducks (*Anas platyrhynchos domesticus*) but histopathological analysis revealed that lesions in H5N8-infected ducks were substantially attenuated and appeared to be less invasive than those in inoculated chickens (Zhao et al., 2013; Kim et al., 2014). An important part of the study performed by Kim and colleagues (2014) was a direct comparison of the virulence of two HPAI H5N1 isolates and a HPAI H5N8 isolate in domestic ducks. This suggested that H5N8 was less virulent than H5N1 in

domestic ducks. In experimental studies, H5N1 was less virulent for wild mallards, common teal, gadwall, and wigeon (all *Anas* spp.) than for domestic ducks. The implication is that H5N8 may be more likely to be maintained and spread by wild *Anas* spp. than H5N1. Kang et al. (2014) reported that inoculated wild mallards (*Anas platyrhynchos*) showed no or mild clinical signs (five birds followed up to 14 dpi). Virus replication and virus excretion was higher in H5N8-infected ducks than in H5N1-infected mallard ducks (*Anas platyrhynchos*). Domesticated ducks (*Anas platyrhynchos domesticus*) did become seriously ill and some animals died. Also the common teal from Germany found to be infected with HPAI H5N8 did not show any pathology or signs that its health had been impaired by infection. Further information on the pathogenesis of HPAI H5N8 infection in wild birds, including source, duration and level of virus excretion, as well as clinical impact, is important for risk assessment studies.

Knowledge of the prevalence of HPAI H5N8 infection in wild bird populations will facilitate understanding the risk of transmission from wild birds to poultry and vice versa. Investigation of goose, swan and duck faecal samples and sentinel birds placed in wetlands (Knight-Jones et al., 2010; Globig et al., 2013) could be further considered. More information and guidelines regarding HPAI surveillance in wild birds can be consulted for instance on the OFFLU website.[6]

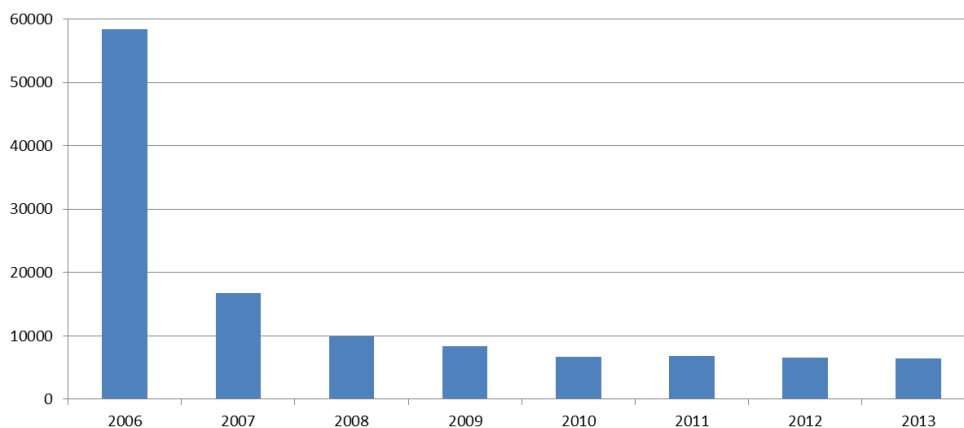**Total Number of Wild Birds Sampled by Passive Surveillance in EU**

Figure 9: Total number of wild birds sampled by passive surveillance in the EU in 2013
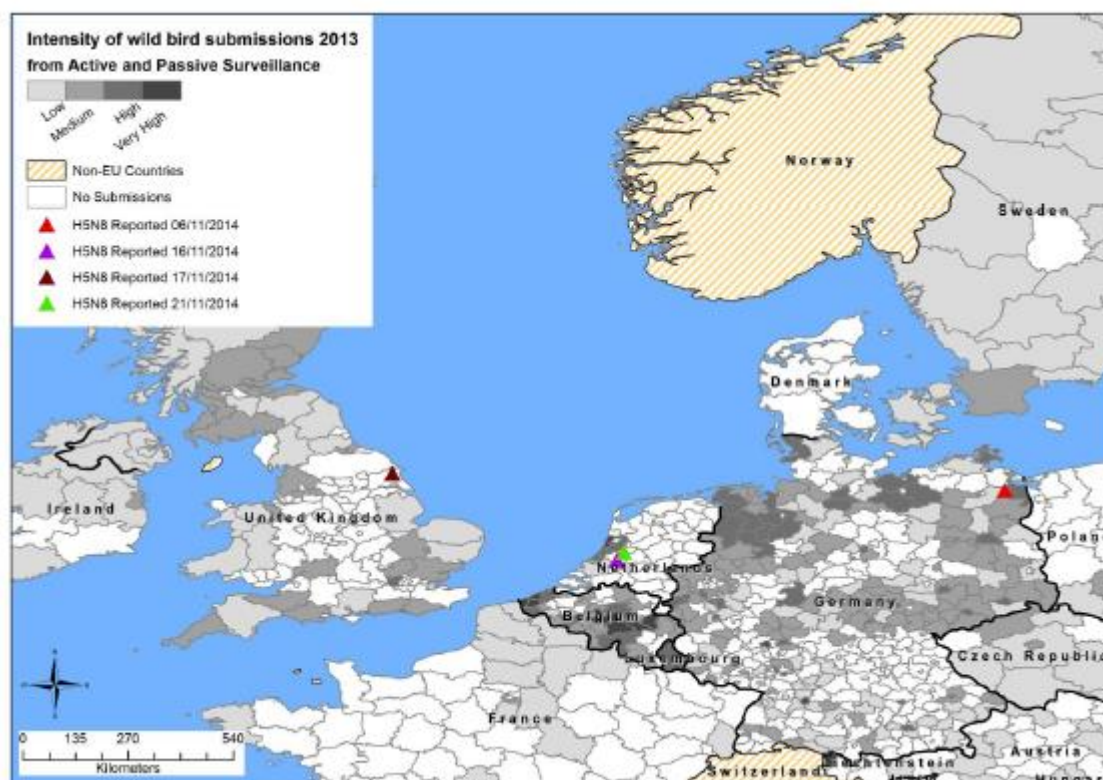
Figure 10: Wild bird sampling density of 2013 based on the EU wild bird surveillance database. Active surveillance is not included at least for some Member States. The locations of the first four affected European holdings are presented.

Table 3 shows data on surveillance in wild birds in 2014 submitted to the European Commission as available on 2nd December 2014. The number of birds tested and detected of H5 LPAI and other LPAI are indicated.

Table 3: Avian Influenza surveillance in Wild Birds 2014 based data submitted to the European Commission wild bird surveillance database by 2 December 2014.

Member State	Full Country Name	Submissions	Data submitted for Q3/Q4	LP_H5 Positives	LP Other Positive	Total Positives
DE	Germany	1435	NO	0	0	0
IT	Italy	844	YES	0	2	2
BE	Belgium	806	NO	0	4	4
ES	Spain	732	NO	0	2	2
HG	Hungary	524	YES	0	0	0
GB	United Kingdom	477	YES	1	4	5
AT	Austria	241	YES	24	38	62
FI	Finland	176	YES	0	1	1
SE	Sweden	113	NO	0	1	1
RO	Romania	109	YES	0	0	0
CY	Cyprus	99	YES	0	0	0
PT	Portugal	90	YES	0	0	0
SI	Slovenia	71	NO	0	1	1
CZ	Czech Republic	69	YES	0	0	0

Member State	Full Country Name	Submissions	Data submitted for Q3/Q4	LP_H5 Positives	LP Other Positive	Total Positives
NL	Netherlands	62	NO	1	0	1
EE	Estonia	33	YES	0	0	0
PL	Poland	24	YES	0	0	0
SK	Slovakia	21	YES	0	0	0
IE	Ireland	16	YES	0	0	0
LT	Latvia	14	NO	0	0	0
GR	Greece	12	YES	0	0	0
DK	Denmark	5	YES	0	0	0
BG	Bulgaria	3	NO	0	0	0
CH	Switzerland	6	YES	0	0	0

The wild bird species targeted for surveillance can be found in a wide range of habitats. Contact between target wild birds and poultry are more likely in regions with wetlands and higher poultry densities (Figure 4). The outbreaks in Germany and The Netherlands have occurred in close proximity of wetland areas with high wild bird densities. The first affected holding in the Netherlands is located near small lakes. The German turkey holding is located 1.3 km from an internationally important wetland for waterbirds. A large group of wild birds were seen on a field in close proximity to the infected premises. There were no dead birds found at the lake close to the affected German holding and all sampled faecal samples of wild birds were negative. The situation is different for the affected UK holding as it is located in an agricultural area with no large wetlands either adjacent to the facility or in the nearby areas. The area around the holding had a low bird population on the day that an ornithological field assessment was carried out and no evidence was reported to suggest the recent presence of large numbers of birds before the infection occurred. Very few waterbirds were present in the 3 km protection zone or 10 km surveillance zone according to a report from experienced ornithologists following a field assessment.

The biosecurity of the European professional poultry holdings is usually moderate to high,[7] but could on average certainly be improved in specific production types (e.g. layer chickens). For indoor poultry holdings, the probability of direct contact between wild birds and poultry is generally low providing that strict biosecurity measures are in place. However, the probability of LPAI virus introduction is higher for (indoor) holdings localized in the proximity of wetlands and with increasing prevalence of LPAI infection in the wild birds present in these wetlands (EFSA, 2005). However, such viruses may evolve into HPAI in poultry holdings. The probability of HPAI being transmitted from wild birds to poultry is more likely when the animals are kept in free-range and backyard holdings or those not having high biosecurity measures. Direct contact between wild birds and poultry is considered very unlikely in the affected UK holding due to the use of a netting system that covered the warehouses. In this holding, wild birds had no access to the feed storage facilities. Poultry were kept indoors in the affected German holding and the affected holdings in the Netherlands. It is puzzling that only indoor kept poultry became infected with HPAI H5N8 in the current outbreaks in Europe and that no outdoor production sites have been affected.

It is clear that following the current HPAI H5N8 outbreaks in Europe, there is a need to strengthen the implementation of biosecurity practices that create barriers in order to reduce the risk of introduction and spread of the virus. Assessing biosecurity procedures at farm and area level with a focus on segregation, cleaning and disinfection, and improving where necessary, is recommended in high risk

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[7] Complying with sector 1 or sector 2 definition of FAO classification of poultry production on the basis of biosecurity level (http://www.fao.org/docrep/013/al674e/al674e00.pdf last accessed 04 December 2014)

areas. The correct use of disinfectants is crucial. More information can for instance be found in the FAO paper on biosecurity for highly pathogenic avian influenza.[8]

4.3. Possible introduction by persons, vehicles, equipment and fomites

Holding owners, workers, veterinarians, vehicles, equipment or any fomite that had contact with contaminated faeces of wild birds or poultry, as well as with carcasses or remains of dead birds can be capable of transmitting the H5N8 virus. Trade in unprocessed poultry manure between Member States is only permitted for manure originating from an area which is not subject to restrictions of avian influenza (Commission Regulation (EC) No 142/2011). The probability of introduction and spread of the H5N8 via persons, vehicles, equipment and fomites is dependent on several factors such as the concentration of virus, stability of the virus under the applicable conditions and characteristics of the fomite (e.g. water content of the material). The possibility of exposure from persons, vehicles and all types of materials to infected wild birds or poultry or their contaminated faeces is not clear but is not expected to be high given that only few wild birds have been found to be infected with H5N8 in Europe and only few poultry holdings have been affected so far. However, given the low sampling effort in wild birds, the actual prevalence of HPAI H5N8 in different wild bird populations in Europe is unknown. HPAI H5N8 introduction into Europe via persons traveling from infected countries in south-east Asia cannot be excluded. The probability of viral transmission between travellers contaminated with HPAI H5N8 and poultry or wild birds is considered to be low, although it cannot be neglected since there are for instance poultry companies with holdings in south-east Asia and Europe.

There is no information available on the stability of H5N8 viruses and extensive variation between strains is likely. For H5N1 and H7N2 for example, it has been reported that these viruses retained their infectivity for a few days in faeces or manure kept at ambient temperature (Lu et al., 2003; Songserm et al., 2005).Tenacity, i.e. resistance of avian influenza virus infectivity against environmental factors outside the host, is influenced by many factors including strain of virus, host origin, environmental matrices (e.g. aerosol, water, faeces, carcass/meat) and environmental conditions (temperature, salinity, pH, protein content, among many others) (EFSA, 2008). In general, avian influenza viruses are more stable with decreasing temperatures and in fresh to brackish salinities (Brown et al., 2007; Keeler et al., 2014). Indirect transmission of avian influenza viruses via water to other water birds which use or drink contaminated water may pose an important factor in infection chains (EFSA, 2008). Influenza viruses are in general vulnerable to disinfectants (EFSA, 2008), although, there are no specific data for H5N8. However, an appropriate biosecurity system is required to prevent virus entry into and virus leaving from a holding since non-avian bridge species such as mice, cats, foxes, rats, dogs and mustelids may act at least as mechanical vectors. It is clear that biosecurity systems should also take into account streams of fomites, waste products and water leaving the holding. There is a lack of data on the role of non-avian transmission vectors in the current HPAI H5N8 outbreaks. Therefore, detailed epidemiological investigations of the affected European farms and a detailed assessment of non-avian transmission vectors that might transport HPAI viruses from south-east Asia to the EU should be undertaken in order to better understand and possibly mitigate the risk of HPAI outbreaks in Europe in the future. Analysis of the Japanese HPAI H5N8 outbreaks would be interesting given the high level of nucleotide identity between HPAI H5N8 viruses currently circulating in Europe and Japan.

This route of infection becomes more likely in holdings with a lower level of biosecurity. The commercial poultry holdings in Europe have in general a moderate to high biosecurity level, but breaches in biosecurity cannot be excluded and improvements can certainly be made. In the German and UK holdings the birds were kept indoors with netting to prevent the entry of wild birds. These two holdings were operating decontamination procedures for vehicles and people entering the holding, while also the feed on the holding was kept under controlled conditions which prevent access by wild birds. The investigations in the holding in the UK indicates that workers may have links to other

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[8] ftp://ftp.fao.org/docrep/fao/011/i0359e/i0359e00.pdf (last accessed on 04 December 2014)

holdings and the holding is part of a larger operation including holdings in other countries, including trade links with Germany, China and the Republic of Korea. Tap water has been used as drinking water, which has a lower probability to be contaminated than for instance surface water.

4.4. Possible introduction by movement of live animals or animal-derived products

Infected live poultry or wild captured birds are potential agents for introduction of avian influenza especially when they are in their incubation period of HPAI, infected with LPAI or of a species that does not show overt clinical signs (e.g. duck species). Day old chicks and hatching eggs are regarded as a low risk although HPAI infections cannot be entirely excluded (EFSA, 2008).

Intra-community trade in, and imports from, third countries of live poultry and hatching eggs should only originate from establishments which fulfil the requirements described in Council Directive 2009/158/EC. Therefore, live poultry or hatching eggs should only be imported from third countries in which avian influenza is a notifiable disease, and when it is free from avian influenza. Similarly, absence of avian influenza needs to be proven when importing other captive birds.[9,10] Within the European Union, holdings also need to be free from avian influenza and poultry may not be transported through areas infected with avian influenza unless by trunk road or rail. Therefore imports during the high risk period (before disease is confirmed and safeguard measures are put in place) and/or illegal importations would pose a risk. The finding of an H5N1-infected exotic bird in a Belgian airport some years ago and more recently at Vienna airport[11] illustrates that this route of virus introduction can occur (Van Borm et al., 2005), although the lack of data impede an evidence-based risk estimation.

Importation of fresh meat has a risk of introducing of avian influenza through products of animal origin with emphasis on duck meat due to the fact that the disease might not have been apparent before slaughter.[12] Eggs for consumption may become infected with HPAI H5N1 during early stages of infection (sick birds will usually stop producing eggs) or in asymptomatic birds; whether the same situation could occur during HPAI H5N8 infection is not known. The probability of poultry exposure to fresh avian meat or eggs is expected to be low and will depend on the likelihood that illegal swill feeding occurs and secondly, whether this swill contains raw scraps or is prepared from kitchen waste that has undergone some form of heat treatment for preparation as food (EFSA, 2008; Harder et al., 2009). Furthermore, egg or meat products are usually subjected to a form of heat treatment which should inactivate the virus. Temperatures above 60-65°C lead to complete HPAI A(H5N1) virus inactivation within a maximum of five minutes even in protein-rich matrices like meat and whole eggs (EFSA, 2008). Although there are no experimental data available at present, it is expected that H5N8 will have a similar heat-lability. Feathers and down from poultry especially ducks and geese are used as a filling for duvets, pillows, thermal clothing and other textiles. The feathers are removed from the carcases after slaughter, or harvested from live birds. The most likely reason that feather or down would be infective is due to contamination with infective faeces or other body fluids (EFSA, 2008). According to current Regulation (EC) No. 142/2011 imported feathers and down should have been treated to ensure that no avian influenza virus remains. Animal by-products and derived products not intended for human consumption (but for instance as feed component) must always be processed and should only be imported from countries free from avian influenza infections as described in Regulation (EC) No. 142/2011. Contaminated packaging materials and trays pose a far greater risk.

Further, previous assessments highlighted the significant illegal importation of poultry products to the EU which provide another risk factor. For example, in the UK in 2003 there were 570 seizures of

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[9] "Other captive bird" refers to any bird other than poultry that is kept in captivity for any reason including those that are kept for shows, races, exhibitions, competitions, breeding or selling.

[10] http://europa.eu/rapid/press-release_IP-07-40_en.htm, accessed 28 November 2014

[11] http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/docs/14062013_hpai_austria_en.pdf; last accessed on 05 December 2014

[12] Food contaminated with influenza viruses does not appear to be a vehicle for infection in humans (EFSA, 2010) doi:10.2903/j.efsa.2010.1629.

10,198 kg of illegally imported poultry meat and other poultry products, and these were thought to be 'only a small proportion of attempted illegal imports' (EFSA, 2005). A further analysis of the current situation is required to estimate the importance of this route of HPAI H5N8 entry into Europe.

In one of the affected, holdings animals had been purchased from a hatchery in the same country (Germany) in July and September 2014. For the same holding it was reported that it is probable that indirect contact with wild birds may have occurred (large flocks of wild birds were observed close to the holding).

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Review of the epidemiological situation of HPAI subtype H5N8 in the world.

- HPAI H5N8 outbreaks in poultry farms have been reported from the Republic of Korea (from January 2014), Japan (from March 2014), China (from May 2014), Germany (from November 2014), United Kingdom (from November 2014) and The Netherlands (from November 2014).

- Field data and preliminary experimental infections indicate that HPAI H5N8 is less pathogenic than HPAI H5N1 in domestic water fowl and aquatic wild birds but not in galliform poultry, and that several wild bird species may by infected with HPAI H5N8 without showing clinical signs.

Epidemiological analysis of the current situation as regards of HPAI subtype H5N8 in Europe in order to assess possible entry routes and in particular the role played by wild birds.

- The entry of HPAI H5N8 into Europe (single or multiple events) and its subsequent further spread within Europe are two separate events which might involve different transmission routes.

- There are no known direct bird migration routes from east Asia to western Europe. Although the movement of individual birds cannot be completely excluded, this is highly improbable.

- It has been hypothesised that long-distance transmission of HPAI viruses could occur as a result of cross-infection between different birds in north Eurasian breeding areas, where wild bird populations from different flyways possibly overlap, but this hypothesis needs further investigation.

- HPAI H5N8 has been detected in one Common Teal (*Anas crecca*) in Germany and two faecal samples of Eurasian Wigeons (*Anas penelope*) in the Netherlands which confirms the presence of the virus in wild bird populations in Europe. However, no signs of increased mortality or morbidity among wild birds have been reported.

- Knowledge of the occurrence of HPAI H5N8 infection in wild bird populations is required in order to better understand the risk of transmission to poultry, which is important in the design of risk management strategies.

- Investigation in the Netherlands using next-generation full genome sequencing with phylogenetic tree analysis suggests separate introductions into four holdings and one between-farm transmission.

- The outbreaks in farms in Europe occurred in facilities which used indoor housing for the poultry; therefore direct contact between wild birds and the farmed birds in the affected holdings was unlikely.

- It is more plausible that indirect introduction of HPAI H5N8 to poultry holdings via humans, vehicles, equipment, fomites, live animals and/or animal-derived products contaminated with virus (for instance in faeces) of infected birds took place.

RECOMMENDATIONS

- Assessing biosecurity procedures at farm and area level with a focus on segregation, cleaning and disinfection, and improving where necessary, is recommended in high risk areas.

- Given the apparent low pathogenicity of HPAI H5N8 for several wild bird species, focussed strategic and proportionate enhancement of both active (targeted) and passive (scanning) surveillance of both living and dead wild birds in the high risk areas would improve understanding the risk of virus transmission to poultry and might facilitate the design of targeted measures to reduce the risk of virus transmission between poultry and wild birds.

- The close collaboration between the national and European laboratories and risk assessment institutions needs to be continued to share data in real time and to ensure timely updated analyses on the evolving situation within the European Union.

- Detailed epidemiological investigations of the affected European farms and a detailed assessment of all transmission routes that might transport HPAI viruses from south-east Asia to the EU should be continued in order to identify the risk of HPAI introduction into Europe and into European poultry holdings.

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APPENDIX

APPENDIX A

Table 4: H5N8 confirmed cases in wild birds

Species[13]	Common names	Family	Distribution size (breeding/resident)[14]	Geographical distribution	Status in UK, DE, NL	Full migrant	Location	Date
Anas formosa Georgi, 1775	Baikal teal, Siberische taling, Baikalente; Gluckente	Anatidae	2,350,000 km²	Breeds in eastern Siberia, Russia and occurs on passage in Mongolia and North Korea. It winters mainly in Japan, South Korea, which now holds the majority of the wintering population, and mainland China, and it is a rare winter visitor to Taiwan (China) and Hong Kong (China).	Rare vagrant	Y	Republic of Korea, Jeonbuk, (Dongrim reservoir) Republic of Korea; Chungam	17-23/01/2014 22-23/01/2014
Anser fabalis Latham, 1787	Bean goose, Taiga bean goose, Taigarietgans, Rietgans; Saatgans	Anatidae	3,990,000 km²	This species has an extremely large range. Wide spread in Europe. This species is strongly migratory and travels between breeding grounds in the high Arctic to wintering grounds in the temperate and subtropical zones (del Hoyo et al. 1992).	Native	Y	Republic of Korea, Seoul, Gwacheon City Republic of Korea, Jeonbuk, (Dongrim reservoir) Republic of Korea, Gyeonggi	11/02/2014 19/01/2014-01/02/2014 01/02/2014
Anser albifrons (Scopoli, 1769)	greater white-fronted goose; white-fronted goose; Blässgans; kolgans	Anatidae	1,590,000 km²	This species has an extremely large range. This species is fully migratory (del Hoyo et al. 1992), travelling in stages via several stop-over sites between separate breeding and wintering grounds (Madge and Burn 1988).	Native	Y	Republic of Korea, Gyeonggi	28/01/2014
Anas platyrhynchos Linnaeus, 1758	Mallard, wilde eend, Stockente	Anatidae	22,500,000 km²	This species has an extremely large range. Wide spread in Europe. In temperate regions breeding populations of this species are sedentary or dispersive, often making local movements during severe weather (Scott and Rose 1996). Other populations are fully migratory with females and juveniles leaving the breeding grounds in the western Palearctic from September and returning as early as February (Kear 2005)	Native	Y	Republic of Korea, Jeonnam China, Shanghai Shi, Shanghai city Republic of Korea, Chollabuk-do Republic of Korea, Chungcheongbuk-do	27-29/01/2014 01/12/2013 01/12/2013 February 2014
Ardea alba Linnaeus, 1758	great egret; great white egret; Silberreiher: grote zilverreiger	Ardeidae	57,200,000 km²	This species has an extremely large range. All populations of this species undergo post-	UK: Vagrant, DE	Y	Republic of Korea, Chollabuk-do, Jeonju	10/03/2014

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[13] EU-NOMEN http://www.eu-nomen.eu/portal/
[14] Bird Life International http://www.birdlife.org/datazone/species/search

Species[13]	Common names	Family	Distribution size (breeding/resident)[14]	Geographical distribution	Status in UK, DE, NL	Full migrant	Location	Date
				breeding dispersive movements (del Hoyo et al. 1992). Populations breeding in the tropics are sedentary (del Hoyo et al. 1992) or partially migratory (in relation to rainfall) (Brown et al. 1982), whereas Palearctic and Nearctic populations are migratory (Flint et al. 1984, del Hoyo et al. 1992).	and NL: Native		City	
Fulica atra Linnaeus, 1758	common coot; coot; Eurasian coot; meerkoet, Belche; Blässhuhn; Blässralle; Blesse; Blesshuhn; Blessralle; Böichn; Bölle; Duckente; Huhent; Hurbel; Lietze; Rohrhuhn; Taucherli; Wasserhuhn; Zappe	Rallidae	22,300,000 km^2	This species has an extremely large range. Wide spread in Europe. Most populations in warm and temperate regions are resident, often making nomadic dispersive movements according to changing water levels and seasonal rainfall. Populations in northern Eurasia are fully migratory however, migrating on a broad front through continental Europe and across the Sahara. Southward movements occur from mid-August to November, with the return passage occurring from late-February to May (Taylor and van Perlo 1998, del Hoyo et al. 1996, Urban et al. 1986).	Native	Y	Republic of Korea, Jeonbuk, (Dongrim reservoir)	22/01/2014
Anas crecca Linnaeus, 1758	common teal; Eurasian teal; green -winged teal; teal; Krickente; Kriekente; wintertaling	Anatidae	26,400,000 km^2	This species has an extremely large range. Northern breeding populations of this species are highly migratory (Madge and Burn 1988) although populations in more temperate regions are sedentary (del Hoyo et al. 1992) or locally dispersive (Scott and Rose 1996). The species breeds from May onwards (Madge and Burn 1988) in single pairs or loose groups (del Hoyo et al. 1992). After the post-breeding moult migratory populations of the species migrate south, the peak of the autumn migration occurring between October and November (Scott and Rose 1996). It returns to the breeding areas from late-February onwards (peaking March-April) (Scott and Rose 1996). Outside of the breeding season the species forms large concentrations, with large flocks of 30-40 and sometimes hundreds of individuals gathering at winter roosting sites (Brown et al. 1982, Madge and Burn 1988).	Native	Y	Germany,Mecklenburg-Western Pomerania, Isle of Rügen / Republic of Korea; Chungam	17/11/2014 / 08/02/2014
Aythya ferina (Linnaeus 1758)	common pochard; pochard; Tafelente; tafeleend	Anatidae	11,900,000 km^2	This species has an extremely large range. Northern populations of this species are highly migratory (Scott and Rose 1996, Snow	Native	Y	Japan, Koto District, Tokyo	13/11/2014

Species[13]	Common names	Family	Distribution size (breeding/resident)[14]	Geographical distribution	Status in UK, DE, NL	Full migrant	Location	Date
				and Perrins 1998), with those breeding in the milder parts of western or southern Europe (Snow and Perrins 1998) being sedentary (del Hoyo et al. 1992, Scott and Rose 1996, Snow and Perrins 1998) or only making short-distance dispersal movements (del Hoyo et al. 1992, Scott and Rose 1996, Snow and Perrins 1998) governed by harsh weather conditions (Scott and Rose 1996).				
Anas Penelope Linnaeus	Eurasian wigeon; widgeon; wigeon; smite; Pfeifente	Anatidae	No information found	No information found	Native	No information found	The Netherlands, Zuid Holland, Kamerik	01/12/2014
Cygnus columbianus	Bewick's swan; Bewic's swan; tundra swan; Pfeifschwan; fluitzwaan	Anatidae	3,630,000 km^2	This species has an extremely large range. This species is fully migratory and travels on a narrow front via specific routes using well-known stop-over sites (Madge and Burn 1988) between its Arctic breeding and temperate wintering grounds (del Hoyo et al. 1992).	Native	Y	Japan, Simane, Yasugisi

Republic of Korea, Jeonbuk | 03/11/2014

06/02/2014 |
| *Anas zonorhyncha* | Spot-billed duck, Eastern Spot-billed duck, Chinesische Fleckschnabelente, Chinese Vlekbekeend | Anatidae | No information found | Breeds in NE Asia, winters to S China, Taiwan and the Philippines (http://avibase.bsc-eoc.org/avibase.jsp; access 04/12/2014) | No information found | No information found | Republic of Korea; Chungam | 08/02/2014 |
| Not applicable | Unidentified wild duck | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Japan, Tottori, Tottori City

Japan, Simane, Nagara Town | 18/11/2014

18/11/2014 |
| Not applicable | Unidentified wild geese | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Republic of Korea, Chollabuk-do, Gochang-gun Donglim | 17/01/2014 |
| Not applicable | Unidentified waterfowl | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Republic of Korea, Jeollabuk-do | February 2014 |

GLOSSARY AND ABBREVIATIONS

GLOSSARY

Avian influenza	An infection of poultry or other captive birds caused by any influenza A virus: (a) of the subtypes H5 or H7; or (b) with an intravenous pathogenicity index in six-week old chickens greater than 1.2.
Biosecurity	Implementation of practices that create barriers in order to reduce the risk of the introduction and spread of disease agents.
Captive bird	Any bird other than poultry that is kept in captivity for any reason other than birds that are kept in captivity for the production of meat, eggs, other products or breeding programmes, but including those that are kept for shows, races, exhibitions, competitions, breeding or selling.
Highly pathogenic avian influenza	An infection of poultry or other captive birds caused by: (a) avian influenza viruses of the subtypes H5 or H7 with genome sequences codifying for multiple basic amino acids at the cleavage site of the haemagglutinin molecule similar to that observed for other HPAI viruses, indicating that the haemagglutinin molecule can be cleaved by a host ubiquitous protease; or (b) avian influenza viruses with an intravenous pathogenicity index in six week old chickens greater than 1.2.
Holding	Any agricultural or other premises, including hatcheries, circuses, zoos, pet bird shops, bird markets, and aviaries, where poultry or other captive birds are being bred or kept. However, this definition does not include slaughterhouses, means of transport, quarantine facilities and centres, border inspection posts and laboratories authorised by the competent authority to hold avian influenza virus.
Low pathogenic avian influenza	An infection of poultry or other captive birds caused by avian influenza viruses of subtypes H5 or H7that do not come within the definition of HPAI.
Other captive bird	Any bird other than poultry that is kept in captivity for any reason including those that are kept for shows, races, exhibitions, competitions, breeding or selling.
Poultry	All birds that are reared or kept in captivity for the production of meat or eggs for consumption, the production of other products, for restocking supplies of game birds or for the purposes of any breeding programme for the production of these categories of birds.
Wild bird	A free-living bird which is not kept on any holding.

ABBREVIATIONS

AI	Avian influenza
dpi	Days post infection
EU	European Union
HA	Haemagglutinin
HPD	Highest posterior density
HPAI	Highly pathogenic avian influenza
LPAI	Low pathogenic avian influenza