

1 **Role for migratory wild birds in the global spread of avian influenza H5N8**

2
3 The Global Consortium for H5N8 and Related Influenza Viruses*†

4 **Abstract**

5 Avian influenza viruses affect both poultry production and public health. A subtype H5N8 (clade
6 2.3.4.4) virus, following an outbreak in poultry in South Korea in 2013/2014, rapidly spread worldwide
7 in 2014/2015. Our analysis of H5N8 viral sequences, epidemiological investigations, waterfowl
8 migration, and poultry trade showed that long-distance migratory birds can play a major role in the
9 global spread of avian influenza viruses. Further, we found that the haemagglutinin of clade 2.3.4.4
10 virus was remarkably promiscuous, creating reassortants with multiple neuraminidase subtypes.
11 Improving our understanding of the circumpolar circulation of avian influenza viruses in migratory
12 waterfowl will help to provide early warning of threats from avian influenza to poultry, and potentially
13 human, health.

14 15 **One Sentence Summary**

16 Interdisciplinary analysis showed a major role for long-distance migratory birds in the global spread of
17 avian influenza virus H5N8 via (sub)arctic breeding areas.

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18 In 2014, highly pathogenic avian influenza (HPAI) virus of the subtype H5N8 caused disease
19 outbreaks in poultry in Asia, Europe and North America (1–3). Avian influenza viruses are a threat
20 both to global poultry production and to public health; they have the potential to cause severe disease
21 in people, and to adapt to transmit efficiently in human populations (4). This was the first time since
22 2005 that a single subtype of HPAIV had spread over such a large geographical area, and that a
23 Eurasian HPAI virus had spread to North America. The rapid global spread of HPAI H5N8 virus
24 outbreaks raised the question by which routes the virus had been transmitted.

25

26 The segment encoding for the haemagglutinin (HA) surface protein of the HPAI H5N8 viruses is a
27 descendant of the HPAI H5N1 virus (A/Goose/Guangdong/1/1996), first detected in China in 1996 (5).
28 Since then, HPAI H5N1 viruses have become endemic in poultry populations in several countries.
29 The H5 viruses have developed new characteristics by mutation and by reassortment with other avian
30 influenza (AI) viruses, both in poultry and wild birds. In 2005-2006, HPAI H5N1 spread from Asia to
31 Europe, the Middle East, and Africa during the course of a few months. While virus spread
32 traditionally had been attributed to transport of infected poultry, infected poultry products, or HPAI-
33 virus-contaminated materials, several observations in the 2005-2006 epidemic suggested that wild
34 birds also might have carried the virus to previously unaffected areas (6).

35

36 A HPAI H5N8 virus with genes from viruses of the influenza A (H5N1) A/Goose/Guangdong/1/1996
37 lineage was first detected in birds at live bird markets in China in 2010 (1). This HPAI H5N8 virus was
38 a reassortant virus with the HA gene segment from HPAI H5N1 virus and other gene segments from
39 multiple other AI viruses circulating in eastern China (1), and is now categorized as HPAI H5 virus
40 clade 2.3.4.4 (7). This clade is unusually promiscuous and has been found in combination with six
41 different neuraminidase (NA) segments, and multiple H5Nx viruses may be circulating at the same
42 time and in the same region (8, 9). The propensity of HPAI H5 virus clade 2.3.4.4 to form novel
43 subtypes capable of rapid, global spread is a major concern.

44

45 HPAI H5N8 virus caused a large avian influenza outbreak in poultry in South Korea in the winter of
46 2013/2014, and subsequently spread to Japan, North America, and Europe, causing outbreaks there
47 between autumn 2014 and spring 2015 (**Table S1**). However, it is not clear by which routes HPAI
48 H5N8 virus spread so rapidly around the world. Although there have been reports on parts of these
49 outbreaks (1, 2, 10) and speculation on possible routes of transmission (3), no comprehensive global
50 analysis has yet been performed.

51

52 The goal of this study was to analyse the available genetic, epidemiological and ornithological data for
53 evidence of the relative contributions from poultry trade and from wild bird movements (3, 6) for the
54 global spread of clade 2.3.4.4 during 2014-2015. For this purpose, we performed phylogeographic
55 analysis of HPAI H5N8 viruses detected in wild birds and poultry from this global outbreak. In
56 addition, we analysed migration patterns of wild birds found infected with HPAI H5N8 virus,

57 epidemiological investigations of HPAI H5N8 virus outbreaks, and poultry trade records from
58 countries where HPAI H5N8 virus was reported (11).

59

60 Initial phylogenetic analysis was performed using HA sequences from HPAI H5 clade 2.3.4.4 viruses
61 of poultry and wild birds from around the world between 2004 and 2015, including subtypes H5N1,
62 H5N2, H5N3, H5N5, H5N6 and H5N8. From 2004 to 2012, clade 2.3.4.4 viruses were circulating
63 predominantly in Eastern Asia (China) with some transmission to South Eastern Asia (**Figures 1 and**
64 **S1**). During this period, transmission involving domestic anseriforms (ducks and geese) appears to
65 dominate, although some contribution from domestic galliforms (chickens and turkeys) and short-
66 distance migratory anseriforms (e.g. mallard ducks) is also evident (**Figure 1**). Unlike H5 segments
67 from other clades, which are mostly found as H5N1, the HPAI H5 segment of the clade 2.3.4.4
68 viruses reassort frequently, acquiring NA segments from co-circulating low pathogenic avian influenza
69 (LPAI) subtypes, including N5 (from 2006-2010), N2 (from 2008-2012), and N8 (from 2010), and more
70 recently N6 (from 2013) (8). To indicate the host species and regions in which the reassortments are
71 thought to have occurred, a reassortment measure was calculated using the number of branches in
72 the posterior set of phylogenetic trees for which the NA-subtype changed whilst the host species and
73 region traits remained the same (normalized by branch lengths). This measure suggests that most of
74 the observed reassortants were generated in domestic anseriforms (**Figure S2**), and particularly
75 domestic anseriforms in Eastern Asia (China) within the time period 2004-2012 (**Figure S3**).

76

77 The time to the most recent common ancestor (TMRCA) for the HA segment of all clade 2.3.4.4 HPAI
78 H5N8 sequences was estimated as June 2010 (95% highest posterior density (HPD): Jan-Oct 2010);
79 the TMRCA for the corresponding NA segments was similar (Sep 2010, 95% HPD: Apr-Dec 2010).

80 Clade 2.3.4.4 HA H5N8 sequences were found in two subclades (**Figure 1**). The smaller and earlier
81 subclade (a in **Figure 1**) contained the first sequenced 2.3.4.4 HPAI H5N8 virus
82 (A/Duck/Jiangsu/k1203/2010[H5N8]). The larger and more recent subclade (b in **Figure 1**) contained
83 sequences from outbreaks in South Korea and other countries included in this study, and which
84 caused multiple HPAI outbreaks in 2014 and 2015 globally. The TMRCA of subclade b was
85 September 2013 for both HA (95% HPD: Jul-Nov 2013) and NA (95% HPD: May-Nov 2013).

86 Consistent with earlier findings (1, 10), the phylogeny indicates that HPAI H5N8 was introduced into
87 South Korea by long-distance migrant anseriforms that acquired it from the pool of HPAI H5 viruses
88 circulating in domestic anseriforms in Eastern Asia (China), although we formally cannot exclude the
89 possibility that HPAI H5 viruses were circulating in unsampled locations (**Figure 1**).

90

91 Distinct, well-supported clades were identified in South Korea, likely originating in the transmission of
92 HPAI H5N8 viruses from long-distance migrants to other wild and domestic birds (10). One clade (c in
93 **Figure 1**) was ancestral to the European outbreak and another (d in **Figure 1**) was ancestral to the
94 North American outbreak. Again, we cannot exclude the possibility that viruses from these subclades
95 were present in unsampled locations.

96

97 More detailed phylogenetic analyses, using only clade 2.3.4.4 H5N8 HA sequences with location
98 coordinates, showed that the virus spread along two main long-distance migration routes: one from
99 the east Asia coast/Korean peninsula, north to the Arctic coast of the Eurasian continent, then west to
100 Europe; and the other north from the Korean peninsula, then east across the Bering Strait, and south
101 along the north-west coast of the North American continent to Canada and the USA (**Figure 2** and
102 **Movie S1**). The reconstruction did not indicate any spread between Europe and North America. The
103 TMRCA for European HA segments was August 2014 (95% HPD: Jul-Oct 2014), and September-
104 October 2014 (95% HPD: Aug-Nov 2014) for the North American HA segments (**Table S2a-b**). Similar
105 results were found from analysis of the NA segments (**Table S2c-d**). There were also four separate
106 introductions into Japan, the first estimated around February 2014 (ancestral date of single virus
107 A/Chicken/Kumamoto/1-7/2014), and then three more, all with TMRCA in October-November 2014.
108 The sequences from one of the Japanese introductions were most closely related to sequences from
109 Taiwan, and those from another to the Russian (A/wigeon/Sakha/1/2014) and European sequences.

110

111 The phylogenetic data were also used to infer the ancestral host categories of the most recent
112 common ancestor of the European and North American outbreak sequences, thus providing evidence
113 for which host type had introduced the viruses into those areas (**Figures 3, S4 and S5, Table S2**).

114 The most likely ancestral host category for the North American outbreak for both HA and NA
115 segments was long-distance migrants (HA: 66% and NA: 84%). A similar result was obtained for
116 Europe (HA: 66% and NA: 70%).

117

118 Several wild bird species with known HPAI H5N8 sequences were long-distance migrants at different
119 stages of their migratory cycle depending on time and place found (**Table S3**): five of the nine species
120 found in South Korea in winter 2013/2014 were long-distance migrants at their wintering sites or on
121 spring migration. Both in North America and Europe, two of the four species found in winter
122 2014/2015 were long-distance migrants at their wintering sites or on autumn migration (11)(**Tables S4**
123 **and S5, Figure S6**).

124

125 The April 2014 HPAI H5N8 virus outbreak in Japan had different characteristics to the later outbreaks
126 in North America and Europe. The Japan outbreak was the only one that was contemporaneous with
127 the outbreak in South Korea and no wild birds were found positive for HPAI H5N8 virus in Japan
128 during that outbreak.

129

130 Qualitative analysis of data from outbreak investigations on affected poultry farms in North America,
131 Europe and Japan (11) (**Table S6**) showed that the likelihood of virus introduction via contaminated
132 water, feed and poultry was negligible (Germany). Furthermore, no links between the outbreaks in
133 one country and those in other countries could be attributed to personnel contacts or trade of live
134 animals, feed, or products of animal origin (Germany, The Netherlands, United Kingdom, Hungary).
135 Many affected poultry farms were located in areas where wild waterfowl are abundant (Germany, The
136 Netherlands, United Kingdom, Italy, Canada). Direct contact with infected wild birds (USA) or indirect

137 contact with materials (e.g., bedding material, boots, wheels of vehicles) contaminated with wild bird
138 faeces was considered the most likely route of introduction into poultry holdings (USA, Germany, The
139 Netherlands, United Kingdom, Italy). In some outbreaks, the source of infection was unknown or
140 inconclusive (Japan, Hungary).

141

142 We reviewed FAO data (12) for 2011 to 2013 on export and import of live domestic ducks and
143 chickens of affected countries to estimate the risk of spread of HPAI virus from South Korea to other
144 countries via international poultry trade (**Table S7**). Data on the export of live poultry from North
145 Korea and Mongolia, also in East Asia, were not available from FAO. Although all countries (Japan,
146 Canada, USA, Germany, Netherlands, United Kingdom, Italy, Hungary) where HPAI H5N8 virus
147 emerged between November 2014 and February 2015 imported live chickens and live domestic
148 ducks in 2013, South Korea reported the export of a low number of live chickens and no export of live
149 domestic ducks, although unreported cross-border trade cannot be excluded. Nevertheless, based on
150 these data, it seems unlikely that international trade in live poultry played a major role in the long-
151 distance spread of South Korean clade HPAI H5N8 virus during the winter of 2014/2015.

152

153 Our analysis, using four different sources of data, indicates that the main routes of large-scale
154 geographical spread of HPAI H5N8 virus were most probably via long-distance flights of infected
155 migratory wild birds, first in spring 2014 from South Korea or other unsampled locations in the region
156 to northern breeding grounds, and then in autumn 2014 from these breeding grounds along migration
157 routes to wintering sites in North America and Europe.

158

159 Recognition of a likely role of wild birds in the spread of HPAI reinforces the need to improve
160 biosecurity on poultry farms and to exclude wild birds from the immediate vicinity of poultry farms.
161 Culling wild birds, draining or disinfecting wetlands would not be effective because these viruses
162 disseminate on rapid time scales over very large distances, making reactive interventions of this kind
163 impractical and ineffective, as well as contravening commitments made by signatory countries to the
164 Convention on Migratory Species and the Ramsar Convention on Wetlands.

165

166 The potential role of wild birds in the circumpolar circulation of influenza viruses does point to the
167 need to increase our knowledge about the connectedness at the vast circumpolar (sub)arctic breeding
168 areas between migratory waterfowl populations originating from different wintering areas. Surveillance
169 of waterfowl at the crossroads of migratory flyways to wintering areas in Europe, Asia, and North
170 America would inform epidemiological risk analysis and provide early warning of specific HPAI threats
171 to poultry, and potentially human, health.

172

173 **References and Notes**

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206

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226

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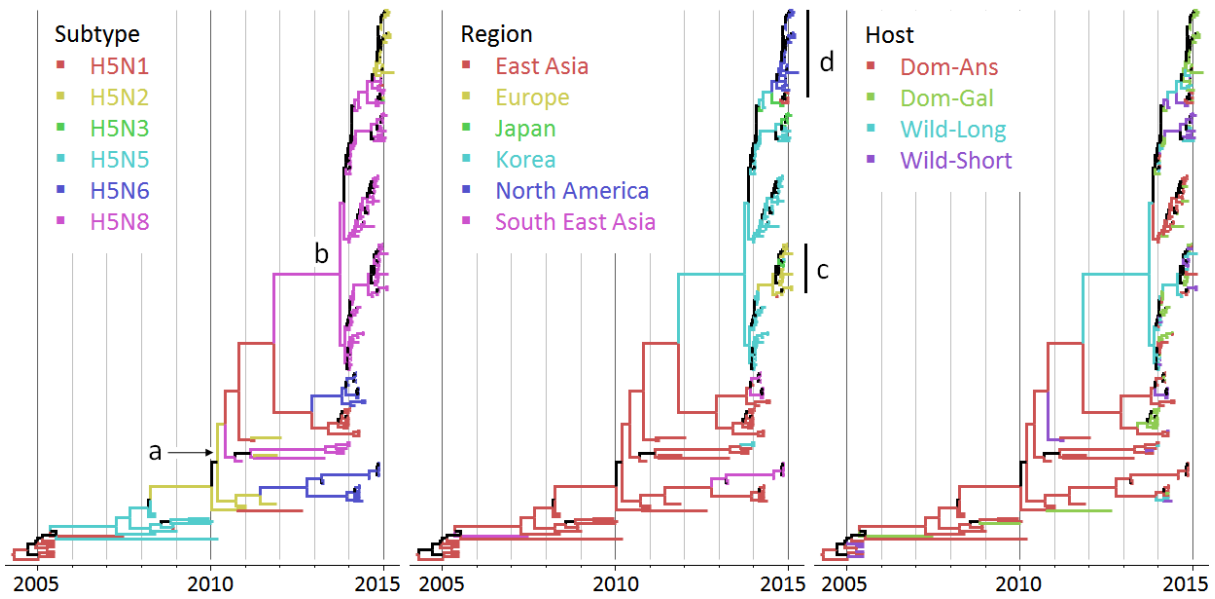
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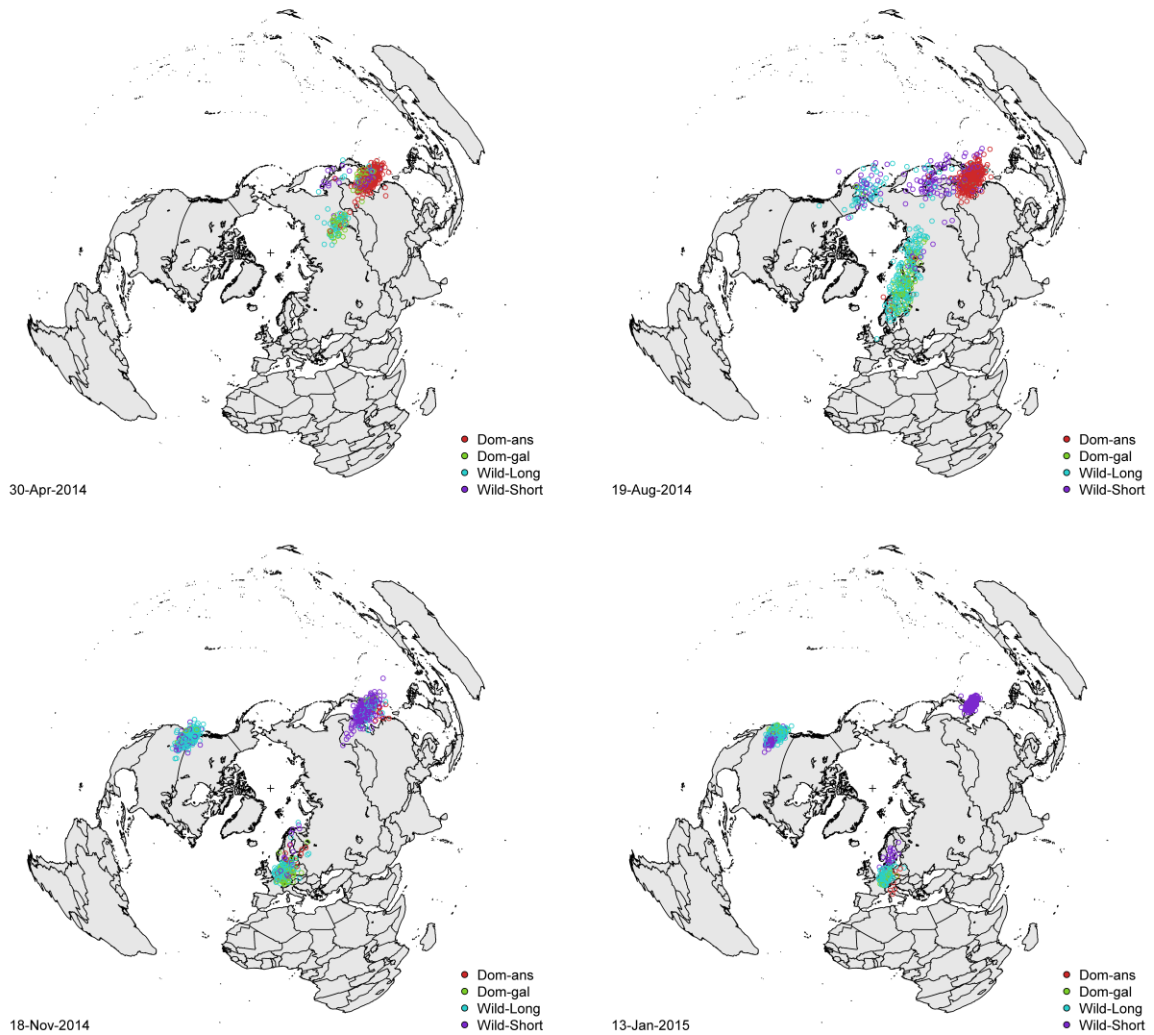
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Figure 1. Maximum clade credibility (MCC) time scaled phylogenetic tree of multi-subtype HA sequences colored by subtype, region and host-type traits. The clades marked a and b contain H5N8 sequences, and c and d contain sequences from Europe and North America, respectively. The displayed MCC tree was obtained from a posterior set of trees inferred using BEAST (13) with the SRD06 nucleotide substitution model, uncorrelated relaxed clock model and constant population size tree prior. The branches are colored according to the most probable ancestral trait, and ancestral traits were inferred by a symmetric (subtype and region) or asymmetric discrete trait model (host-type) upon the posterior tree set (14). Host-types are Dom-Ans (red): domestic anseriform birds, Dom-Gal (green): domestic galliform birds, Wild-Long (blue): long-distance migratory wild birds, Wild-Short (purple): short-distance migratory wild birds.

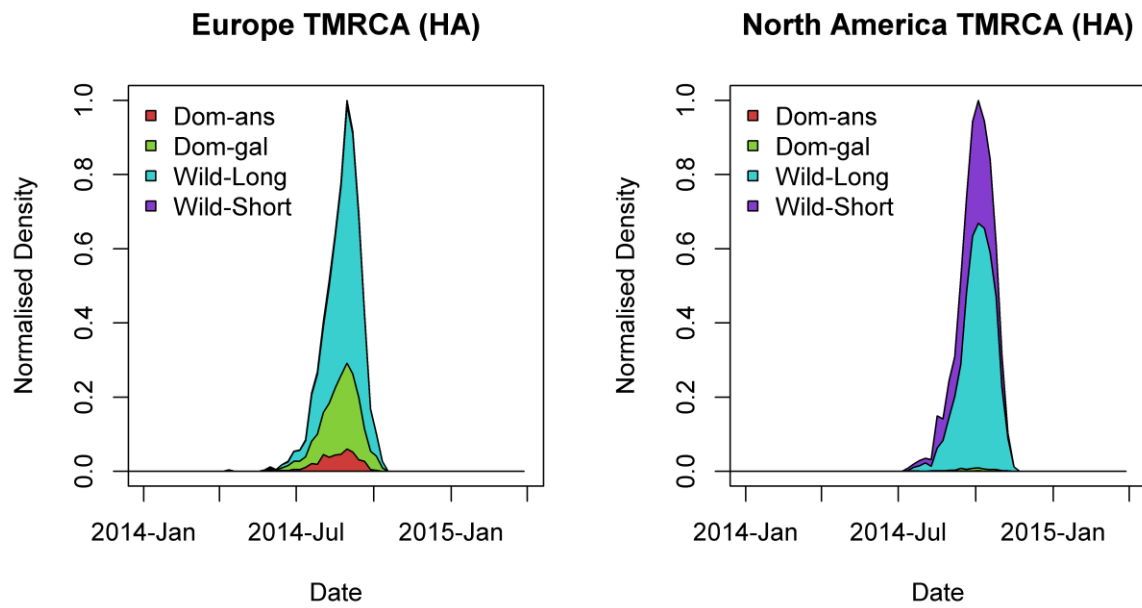


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289 **Figure 2.** Reconstruction of the transmission route using phylogenetic data only from H5N8 HA
 290 sequences. At each time slice, the host-type and location coordinates on the branches of the posterior
 291 set of phylogenetic trees are inferred and plotted as a cloud of points. The host-type was inferred by
 292 discrete trait model (as Figure 1) (14), and the continuous location coordinates were inferred using a
 293 homogeneous Brownian motion diffusion model (15). The map projection used is the azimuthal
 294 equidistant projection, centred on the North Pole, which is marked with a + sign. In this projection, all
 295 points on the map are at proportionately correct distances and at the correct direction from the North
 296 pole. Color key as for Fig. 1; see also Movie 1.

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299

300 **Figure 3.** Posterior distributions of time to most recent common ancestor (TMRCA) of HA sequences
 301 from Europe and North America with H5N8 subtype only, including Host type reconstructions, based
 302 upon a posterior set of phylogenetic trees generated as in Fig 1. Color key as for Fig. 1.

303

304 **Supplementary Materials**

305 Materials and Methods

306 Figures S1-S6

307 Tables S1-S10

308 Movie S1

309 References 16-59