

# Comparison of 2016–17 and Previous Epizootics of Highly Pathogenic Avian Influenza H5 Guangdong Lineage in Europe

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We analyzed the highly pathogenic avian influenza (HPAI) H5 epizootic of 2016–17 in Europe by epidemiologic and genetic characteristics and compared it with 2 previous epizootics caused by the same H5 Guangdong lineage. The 2016–17 epizootic was the largest in Europe by number of countries and farms affected and greatest diversity of wild birds infected. We observed significant differences among the 3 epizootics regarding region affected, epidemic curve,

seasonality, and outbreak duration, making it difficult to predict future HPAI epizootics. However, we know that in 2005–06 and 2016–17 the initial peak of wild bird detections preceded the peak of poultry outbreaks within Europe. Phylogenetic analysis of 2016–17 viruses indicates 2 main pathways into Europe. Our findings highlight the need for global surveillance of viral changes to inform disease preparedness, detection, and control.

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Highly pathogenic avian influenza (HPAI) is a zoonotic notifiable disease that can cause high mortality rates in most domestic poultry and in some wild bird species. Since 2003, HPAI H5 viruses have been circulating in poultry in many countries (1). Periodically these poultry HPAI viruses have been reintroduced into the wild migratory bird population, representing a key risk pathway for its subsequent global spread (1–3). However, the effect of HPAI infection in both wild and domestic birds is variable and often strain-specific. Wild birds, particularly of the orders Anseriformes and Charadriiformes, are natural hosts of low pathogenicity avian influenza (4).

A passive surveillance system of testing wild birds found dead or sick for avian influenza has been in place in European Union (EU) member states since 2005 (Commission Decision 2005/94/EC, replaced with 2010/367/EU), with the objective of timely detection of HPAI subtype H5N1. Laboratory confirmation of HPAI infection following the development of clinical signs (passive surveillance) is the primary method of poultry surveillance in the EU member states, complemented by a serologic active surveillance program (5).

During epidemiologic year 2005–06 (epidemiologic years run from October to September of the next year),

HPAI H5N1 clade 2.2 virus of the Guangdong H5 lineage spread to a number of countries in Europe, infecting poultry and wild bird populations (3). In 2014–15, another virus of the same lineage, HPAI H5N8 clade 2.3.4.4, was introduced into Europe and associated with variable disease severity, including subclinical infection in wild birds and domestic waterfowl (6). This H5N8 virus showed unprecedented intercontinental spread to the United States and Canada and was associated with both wild bird infection and, subsequent to local genetic reassortment, large HPAI H5N2 outbreaks in poultry (7).

In October 2016, a novel HPAI H5 clade 2.3.4.4 virus of the Guangdong lineage was detected in Hungary and was subsequently reported in other countries in Europe, infecting many poultry farms and causing both large-scale and sporadic deaths in wild bird populations. The hemagglutinin (HA) gene of this virus was considered phylogenetically distinct from the previous 2014 clade 2.3.4.4 viruses and was nominally suffixed by A (the 2016 clade) or B (the 2014 clade) (8) but this subclade definition requires verification by the World Health Organization H5 nomenclature group. We describe the epidemiology and genetic characteristics of the 3 major wild-bird mediated epizootics in Europe associated with the Guangdong HPAI H5 lineage.

## Methods

### Epidemiologic Data and Analyses

We collected data from the 3 major HPAI H5 epizootics in Europe: HPAI H5N1 in epidemiologic year 2005–06 (2); HPAI H5N8 in 2014–15; and HPAI H5 in 2016–17. For 2016–17, we collected data through July 31, 2017. We obtained epidemiologic data from the Animal Disease Notification System and the Directorate-General for Health and Food Safety, managed by the European Commission, and from country notifications sent to the EU Reference Laboratory for avian influenza (Animal and Plant Health Agency, Weybridge, UK).

We conducted analyses to describe each epizootic, examined the geographic and temporal spread (epidemic curves), and assessed differences in clinical illness and death rates. For spatial analysis, we grouped countries into 4 regions (North, South-West, South-East, and Central Europe) on the basis of the broad migration patterns of the major migratory water bird species affected by HPAI (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/24/12/17-1860-Techapp1.pdf>) (9–14). A full description of the methods used is presented in the online Technical Appendix.

### Viruses' Sequence Data and Phylogenetic Analyses

We obtained virus HA gene sequence data from countries' submissions to the EU Reference Laboratory and

from GISAID (<http://platform.gisaid.org>.) We performed phylogenetic analyses on HA sequence data from each epizootic separately. We used IQ-TREE version 1.5.5 software (15) to infer maximum-likelihood trees with approximate likelihood ratio test (1,000 replicates) and bootstrap (100 replicates) support values for branches. We down-sampled each dataset using Cluster Database at High Identity with Tolerance to remove sequences with >99.9% sequence identity (16). We performed root-to-tip regression analyses using Tempest version 1.5 on the downsampled datasets (17). Then, we inferred Bayesian phylogenetic trees from each downsampled dataset using BEAST version 1.8.4 to determine the mean substitution rate and TMRCA (time to most recent common ancestor) (18). We annotated the final trees using FigTree version 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). Details of criteria and priors used in the analyses are provided in the online Technical Appendix.

## Results

### Epizootic Size

In 2016–17, a total of 1,108 poultry outbreaks were reported in 21 countries in Europe. Extensive farm-to-farm spread, predominantly in ducks, seemed apparent in France, which had >400 farms affected, and Hungary, with >200 farms infected (19). Conversely, in 2005–06, a total of 230 poultry outbreaks occurred in 6 countries, mostly located in Romania (86%) and Hungary (13%). In 2014–15, only 13 poultry outbreaks were reported in 5 countries. The estimated number of poultry culled was 8 times higher in 2016–17 than in 2005–06 (Table 1).

The number of wild bird detections was substantially different between epizootics: 1,559 incidents in 27 countries in 2016–17, 487 in 18 countries in 2005–06, and only 5 in 3 countries in 2014–15. Almost half of the wild bird incidents reported in all 3 epizootics were in Germany.

### Wild Birds Species and Mass Mortality Events

A total of 49 different wild bird species were reported infected with HPAI H5 virus of the Guangdong lineage in 2016–17, 28 in 2005–06, and 6 in 2014–15 (Table 2,3). Swans (*Cygnus* spp.), particularly mute swans (*Cygnus olor*), were the most frequent species infected in 2005–06 (41% of all wild birds) and 2016–17 (20% of all wild birds). Ducks were the second most common type of wild birds infected. In 2005–06 and 2016–17, tufted duck (*Aythya fuligula*) was the most frequent duck species detected positive (5% of all wild birds). In 2005–06, a total of 28 (6%) mass mortality events (>5 birds dead in 1 location) were reported, whereas 112 (7%) mass mortality events were reported in 2016–17; none were reported in 2014–15 (online Technical Appendix Figure 2). The number of wild

birds found dead by incident was significantly different between epizootics ( $p < 0.001$  by Mann-Whitney U test).

### Type of Poultry Farm and Clinical Manifestations

The types of poultry infected in each epizootic are shown in Table 4. In 2016–17, a large proportion of infected farms (40%) kept ducks. In 2005–06, many affected backyard flocks in Romania (176/230, 77%) had <100 birds, whereas 70% (9/13) of poultry farms infected in 2014–15 had >10,000 birds and >60% in 2016–17 had >1,000 birds (difference in flock size distribution,  $p < 0.001$  by Kruskal-Wallis test). When we excluded Romania from the comparison of flock size, there was no statistical difference in flock size between 2005–06 and 2016–17 (online Technical Appendix Figure 3).

Ducks, geese, turkeys, and broiler chickens on average had higher illness rates in 2005–06 than in the other epizootics (Figure 1). In 2016–17, average mortality rate was lowest in ducks (7%) and turkeys (6%); few farms (<5%) reported a >25% mortality rate. In contrast, 32% of affected broiler farms and 27% of affected layer farms reported mortality rates >25%. In 2005–06, more than half of broiler farms reported mortality rates >25%. When comparing overall estimates, we found the observed poultry illness and death rates to be substantially higher in 2005–06 than in 2016–17.

### Temporal Spread

We determined the epidemiologic curves of the 3 epizootics (Figure 2, panels A–C). In 2016–17, H5 was first detected in Europe in a mute swan in Hungary; the first outbreak in

**Table 1.** Highly pathogenic avian influenza outbreaks by country in 3 epizootics in Europe\*

Country	H5N1 2005–06 epizootic			H5N8 2014–15 epizootic				H5N8 2016–17 epizootic			
	No. poultry infected	No. wild birds infected	No. poultry culled†	No. poultry infected	No. wild birds infected	No. captive birds infected	No. poultry culled†	No. poultry infected	No. wild birds infected	No. captive birds infected	No. poultry culled†
France	1	21	11,700	–	–	–	–	485	51	3	1,529,361
Hungary	29	12	251,948	1	–	–	22,000	238	86	5	2,678,191
Germany	1	220	14,300	5	2	1	58,964	89	738	15	1,150,631
Bulgaria	–	4	–	–	–	–	–	71	13	2	511,832
Poland	–	29	–	–	–	–	–	65	66	–	1,167,282
Romania	197	17	755,372‡	–	–	–	–	45	93	2	2,222
Czech Republic	–	14	–	–	–	–	–	38	39	–	79,308
Italy	–	19	–	1	–	–	31,985	16	6	–	357,049
Spain	–	1	–	–	–	–	–	10	2	–	28,330
Croatia	§	§	§	–	–	–	–	9	12	–	1,546
United Kingdom	–	1	–	1	–	–	6,178	12	23	–	102,849
Netherlands	–	–	–	5	1	–	245,600	8	48	10	202,004
Slovakia	–	2	–	–	–	–	–	8	58	3	351
Greece	–	25	–	–	–	–	–	5	8	–	28,275
Serbia	§	§	§	–	–	–	–	4	20	–	289
Sweden	1	13	692	–	2	–	–	4	30	2	203,053
Austria	–	46	–	–	–	–	–	2	55	1	1,258
Ukraine	§	§	§	–	–	–	–	2	3	1	10,288
Bosnia and Herzegovina	§	§	§	–	–	–	–	1	1	1	148
Denmark	1	26	102	–	–	–	–	1	49	1	69
FYROM	§	§	§	–	–	–	–	1	1	–	438
Belgium	–	–	–	–	–	–	–	2	3	13	4,047
Finland	–	–	–	–	–	–	–	–	15	2	–
Ireland	–	–	–	–	–	–	–	–	10	–	–
Lithuania	–	–	–	–	–	–	–	–	5	–	–
Portugal	–	–	–	–	–	–	–	–	1	–	–
Slovenia	–	28	–	–	–	–	–	–	41	–	–
Switzerland	–	9	–	–	–	–	–	–	87	–	–
Luxembourg	–	–	–	–	–	–	–	–	–	4	–
Totals	230	487	1,034,114	13	5	1	364,727	1,116	1,565	64	8,058,831
Total infected	717			19				2,745			

\*Table includes all reported HPAI H5N8 outbreaks through July 31, 2017. It excludes the new wave of secondary H5N8 outbreaks observed in Italy from the beginning of July 2017 through September 2017, which has different drivers and kinetics with maintenance in the poultry (primarily turkey) population rather than through wild bird introduction. FYROM, the former Yugoslav Republic of Macedonia; HPAI, highly pathogenic avian influenza.

†It is uncertain if for some outbreaks only the number of poultry in one farm building or if the poultry population in the area of the farm were reported. This estimate should be used as an approximation and indicator of impact.

‡One observation contained 600,000 birds, representing the overall population of backyard flocks affected in Romania. This number is an approximation.

§These countries did not submit data to the Animal Disease Notification System in 2005–06; however, there is other evidence of H5N1 incursion in the period.

poultry was detected 11 days later in a turkey farm, also in Hungary. We observed 3 major epidemic peaks on the incidence of poultry outbreaks (Figure 2, panel D): on day 54 (14.9 outbreaks/wk), following large farm-to-farm spread in Hungary; day 79 (12.1 outbreaks/wk) caused by farm-to-farm transmission in France and Bulgaria; and on day 121 (16.9 outbreaks/wk), caused by the large farm-to-farm spread in France and Poland.

In 2005–06 and 2016–17, a peak in wild bird incidents preceded the peak in poultry outbreaks (Figure 2, panel A, C). Statistical analysis of the distribution of the epidemic curves indicates that the 2016–17 outbreak had significantly higher incidence values ( $p < 0.001$  by 2-sample Kolmogorov-Smirnov test) than the other 2 epizootics;

2005–06 had significantly higher values ( $p < 0.001$  by 2-sample Kolmogorov-Smirnov test) than 2014–15. Temporal median of the poultry epizootic was substantially different between epizootics (mean/median distance for 2005–06, 189/223 days; for 2014–15, 33.5/26; for 2016–17, 92/90 days). Seasonal analysis of poultry outbreaks indicates significant differences ( $p < 0.001$  by Pearson  $\chi^2$  test) between epizootics;  $> 50\%$  of poultry outbreaks occurred in May in 2005–06, in November in 2014–15, and in December–February in 2016–17 (Figure 2, panel E).

### Spatial Spread

We mapped a temporal-spatial analysis of the 3 epizootics (Figures 3–5). The data shown in Figure 5, panel B, suggest

**Table 2.** Wild bird species of the orders Podicipediformes, Anseriformes, and Charadriiformes, reported by event in 3 highly pathogenic avian influenza epizootics in Europe

Species group	Species	No. (%) events		
		H5N1 2005–06 epizootic	H5N8 2014–15 epizootic	H5N8 2016–17 epizootic
Rails	Eurasian coot ( <i>Fulica atra</i> )	5 (1)		8 (0.5)
	Crested coot ( <i>Fulica cristata</i> )			1 (0.1)
	Purple swamphen ( <i>Porphyrio porphyrio</i> )	4 (1)		
	Common moorhen ( <i>Gallinula chloropus</i> )	1 (0.2)		2 (0.1)
	Total	10 (2)		11 (1)
Swans	Unspecified	197 (38)	2 (22)	262 (16)
	Mute swan ( <i>Cygnus olor</i> )	92 (18)		344 (20)
	Whooper swan ( <i>Cygnus cygnus</i> )	2 (0.4)		80 (5)
	Total	291 (56)	2 (22)	683 (41)
Ducks	Unspecified	57 (11)		143 (9)
	Northern pintail ( <i>Anas acuta</i> )	2 (0.4)		
	Eurasian wigeon ( <i>Anas penelope</i> )		1 (11)	21 (1)
	Mallard ( <i>Anas platyrhynchos</i> )	4 (1)	1 (11)	43 (3)
	Common pochard ( <i>Aythya farina</i> )	4 (1)		8 (0.5)
	Red-crested pochard ( <i>Netta rufina</i> )			2 (0.1)
	Common goldeneye ( <i>Bucephala clangula</i> )			1 (0.1)
	Greater scaup ( <i>Aythya marila</i> )	2 (0.4)		
	Common merganser ( <i>Mergus merganser</i> )	5 (1)		
	Tufted duck ( <i>Aythya fuligula</i> )	18 (3)		82 (5)
	Eurasian teal ( <i>Anas crecca</i> )		1 (11)	3 (0.2)
	Smew ( <i>Mergus albellus</i> )	1 (0.2)		
	Shelduck ( <i>Tadorna tadorna</i> )			2 (0.1)
	Common eider ( <i>Somateria mollissima</i> )			2 (0.1)
	Total	93 (18)	3 (33)	307 (18)
Geese	Unspecified	30 (6)		94 (6)
	Canada goose ( <i>Branta canadensis</i> )			5 (0.3)
	Barnacle goose ( <i>Branta leucopsis</i> )	1 (0.2)		
	Greater white-fronted goose ( <i>Anser albifrons</i> )			9 (1)
	Lesser white-fronted goose ( <i>Anser erythropus</i> )	2 (0.4)		4 (0.2)
	Greylag goose ( <i>Anser anser</i> )	1 (0.2)		21 (1)
	Red-breasted goose ( <i>Branta ruficollis</i> )	1 (0.2)		
	Bean goose ( <i>Anser fabalis</i> )			1 (0.1)
	Pink-footed goose ( <i>Anser brachyrhynchus</i> )			1 (0.1)
	Total	35 (7)		134 (8)
Gulls	Unspecified	9 (2)		89 (5)
	Great black-backed gull ( <i>Larus marinus</i> )			11 (1)
	Herring gull ( <i>Larus argentatus</i> )	1 (0.2)		28 (2)
	Black-headed gull ( <i>Larus ridibundus</i> )	1 (0.2)	1 (11)	23 (1)
	Lesser black-backed gull ( <i>Larus fuscus</i> )			1 (0.1)
	Common gull ( <i>Larus canus</i> )			2 (0.1)
Total	11 (2)	1 (11)	154 (9)	
Waders	Green sandpiper ( <i>Tringa ochropus</i> )			1 (0.1)
	Eurasian curlew ( <i>Numenius arquata</i> )			1 (0.1)
	Total			2 (0.1)

that, in the first 2 months of the 2016–17 epizootic, 2 different viral incursions may have occurred: one spreading through Hungary, Croatia, Switzerland, and southern Germany, and another spreading in northern Europe (Poland, Denmark, northern Germany, Sweden, and the Netherlands). The 2005–06 epizootic indicated a similar progression pattern, initiating in Romania and spreading up to northern Europe and down to southeastern Europe (Figure 3).

Comparison by region of Europe according to wild bird migratory patterns indicates poultry outbreaks were mostly observed in the South-East and South-West regions in 2005–06 and 2016–17 but in the North in 2014–15 (online Technical Appendix Figure 4). Most wild bird detections were reported in the North and Central regions.

Poultry detections by region were significantly different for the 3 epizootics ( $p < 0.001$  by Pearson  $\chi^2$  test), whereas wild bird detections by region were only significantly different ( $p < 0.001$  by Pearson  $\chi^2$  test) between 2005–06 and 2016–17.

### Phylogenetic Analysis

Genetic analysis of the HA gene for the 2014–15 and 2016–17 epizootics shows the involvement of H5 clade 2.3.4.4 in all cases where data were available (Figure 6). Patterns found in maximum-likelihood trees are largely in agreement with the Bayesian analysis; however, a greater proportion of the clades remain unresolved in the maximum-likelihood trees (Figure 6; online Technical Appendix Figure 8). The

**Table 3.** Wild bird species of orders other than Podicipediformes, Anseriformes, and Charadriiformes reported by event in 3 highly pathogenic avian influenza epizootics in Europe

Species group	Species	No. (%) events		
		H5N1 2005–06 epizootic	H5N8 2014–15 epizootic	H5N8 2016–17 epizootic
Birds of prey	Unspecified	30 (6)		
	Buzzard	1 (0.2)		6 (0.4)
	Eagle			1 (0.1)
	Falcon	1 (0.2)		3 (0.2)
	Hawk	1 (0.2)		3 (0.2)
	Owl	2 (0.4)		4 (0.2)
	Barn owl ( <i>Tyto alba</i> )	1 (0.2)		
	Peregrine falcon ( <i>Falco peregrinus</i> )	1 (0.2)		8 (0.5)
	White-tailed eagle ( <i>Haliaeetus albicilla</i> )			24 (1)
	Common buzzard ( <i>Buteo buteo</i> )	7 (1)		70 (4)
	Rough-legged buzzard ( <i>Buteo lagopus</i> )	1 (0.2)		
	Eurasian eagle-owl ( <i>Bubo bubo</i> )	2 (0.4)		1 (0.1)
	Eurasian sparrowhawk ( <i>Accipiter nisus</i> )			1 (0.1)
	Common kestrel ( <i>Falco tinnunculus</i> )			2 (0.1)
	Northern goshawk ( <i>Accipiter gentilis</i> )			1 (0.1)
Total	47 (9)		124 (7)	
Crows	Unspecified	1 (0.2)		
	Eurasian magpie ( <i>Pica pica</i> )	1 (0.2)		4 (0.3)
	Hooded crow ( <i>Corvus cornix</i> )			3 (0.2)
	Rook ( <i>Corvus frugilegus</i> )			2 (0.1)
	Carrion crow ( <i>Corvus corone</i> )			1 (0.1)
	Common raven ( <i>Corvus corax</i> )			1 (0.1)
	Total	2 (0.4)		11 (1)
Grebes	Great crested grebe ( <i>Podiceps cristatus</i> )	7 (1)		12 (1)
	Little grebe ( <i>Tachybaptus ruficollis</i> )	1 (0.2)		4 (0.2)
	Total	8 (2)		16 (1)
Thrushes	Blackbird ( <i>Turdus merula</i> )			1 (0.1)
	Song thrush ( <i>Turdus philomelos</i> )			2 (0.1)
	Total			3 (0.2)
Pigeons, doves	Wood pigeon ( <i>Columba palumbus</i> )			2 (0.1)
	Collared dove ( <i>Streptopelia decaocto</i> )	1 (0.2)		1 (0.1)
	Rock dove ( <i>Columba livia</i> )		1 (11)	
	Total	1 (0.2)	1 (11)	3 (0.2)
Hérons	Unspecified	2 (0.4)		16 (1)
	Grey heron ( <i>Ardea cinerea</i> )	4 (1)		48 (3)
	Total	6 (1)		64 (4)
Storks	Unspecified	2 (0.4)		
	White stork ( <i>Ciconia ciconia</i> )			3 (0.2)
	Total	2 (0.4)		3 (0.2)
Pelicans	Unspecified. ( <i>Pelcanus</i> spp.)			2 (0.1)
Terns	Common tern ( <i>Sterna hirundo</i> )			2 (0.1)
Cormorants	Great cormorant ( <i>Phalacrocorax carbo</i> )	6 (1)		17 (1)
Other	Unspecified	9 (2)	2 (22)	140 (8)



**Table 4.** Types of poultry on infected farms in 3 highly pathogenic avian influenza epizootics in Europe\*

Type of poultry	H5N1 2005–06 epizootic		H5N8 2014–15 epizootic		H5N8 2016–17 epizootic	
	No. (%) farms	No. with only 1 species	No. (%) farms	No. with only 1 species	No. (%) farms	No. with only 1 species
Ducks			3 (23)	0	495 (44)	433
Geese					113 (10)	81
Ducks and geese	29 (13)	0				
Turkey	5 (2)	1	3 (23)	0	91 (8)	82
Broilers	23 (10)	17	4 (31)	0	93 (8)	48
Laying hens					47 (4)	29
Pigeons					9 (1)	1
Guinea fowl					10 (1)	1
Peacocks					2 (0)	0
Pheasants					8 (1)	5
Quail					2 (0)	1
Ostrich					1 (0)	0
Backyard†	176 (77)	NA				
Unknown			2		360(32)	NA
<b>Total infected farms</b>	<b>230</b>		<b>13</b>		<b>1,116</b>	

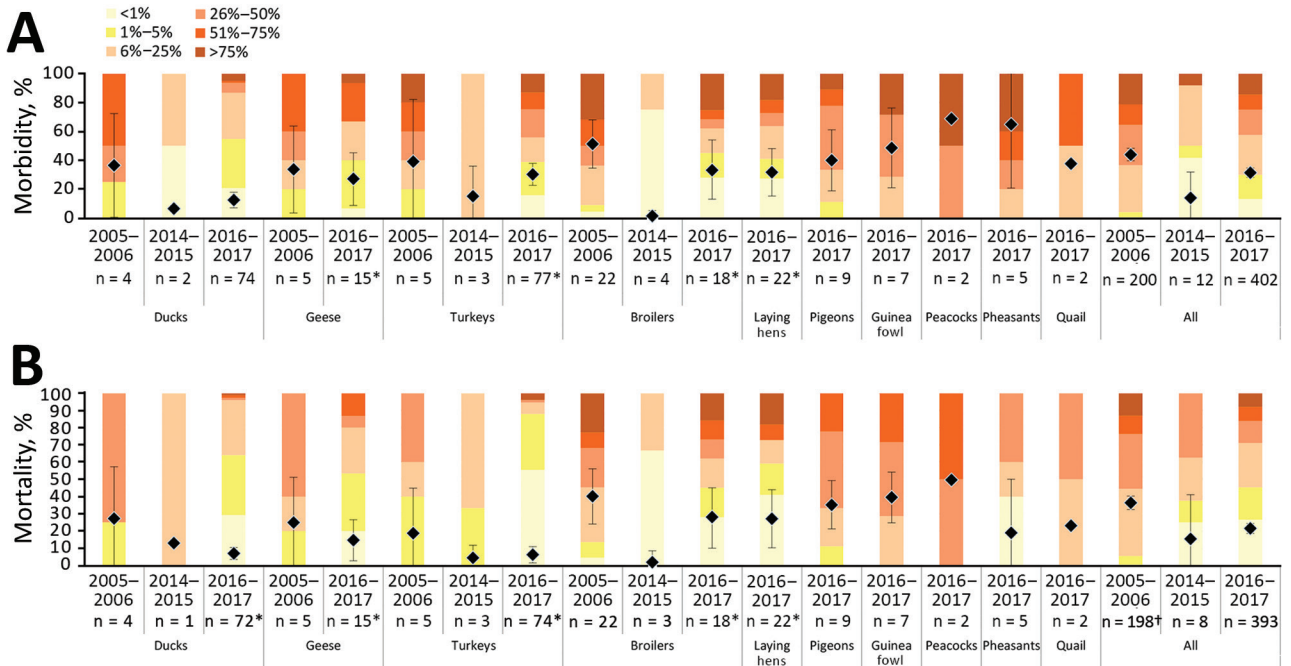
\*NA, not available.

†Backyard represents those households that keep few birds, normally layer hens, for their own consumption. The category was used only in the 2005–06 epizootic.

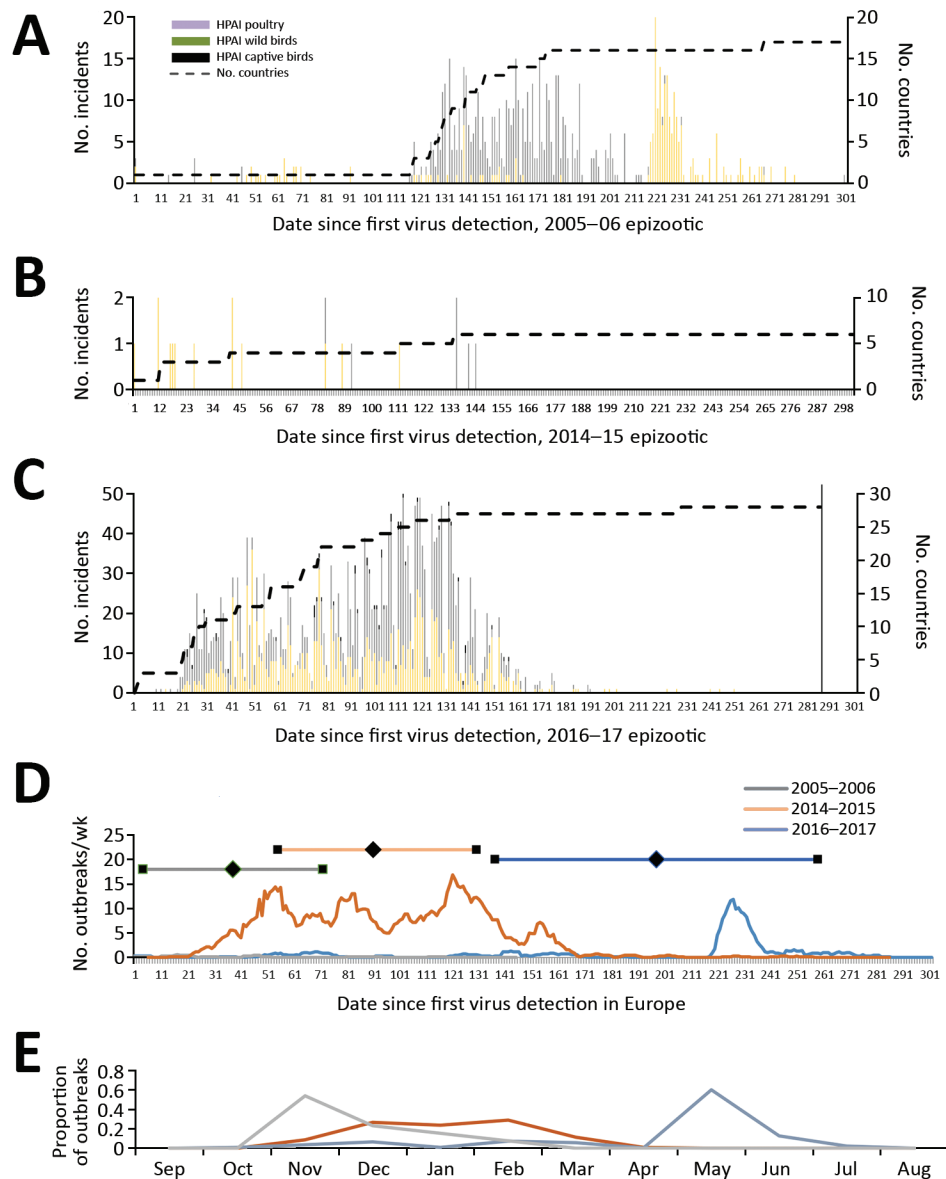
2016–17 viruses form a distinct clade and can be clearly differentiated from the clade 2.3.4.4. viruses present in Europe in 2014–15. In agreement with the geospatial results, analysis of the HA gene of the viruses from the 2016–17 epizootic shows that most originate from a common progenitor (time to most recent common ancestor estimated May 2014–August 2015) (online Technical Appendix Figure 8). However, these viruses differ in their evolutionary pathway thereafter, evolving in 2 co-circulating subclades without clear geographic restriction (time to most recent common ancestor

March 2015–August 2016 [0.9 posterior probability] and November 2014–October 2015 [0.82 posterior probability]). This finding potentially indicates 2 major incursion pathways via wild birds.

We also found smaller clusters and singleton sequences including sequences from European viruses; viruses from 2014–15 form 1 subclade, estimated to have emerged in January–February 2014 (Figure 6; online Technical Appendix Figure 8). The 2005–06 data show viruses in several subclades, but the branching pattern in



**Figure 1.** Morbidity (A) and mortality (B) rates as percentages of populations reported in infected poultry farms during 3 highly pathogenic avian influenza epizootics in Europe, 2005–06, 2014–15, and 2016–17. Years given are epidemiologic years (October through September of the next year). Diamonds with error bars indicate means and 95% CIs. Asterisks indicate farms with unique poultry species used for analysis; dagger indicates large majority of data from backyard farms reported in Romania.



**Figure 2.** Epidemic curve of 3 HPAI H5 virus epizootics in Europe: A) 2005–06 H5N1; B) 2014–15 H5N8; C) 2016–17 H5N8. Years given are epidemiologic years (October through September of the next year). Dashed lines indicate number of countries reporting an HPAI infection since the beginning of the epizootic; vertical line in panel C indicates data collected through July 31, 2017. D) Weekly average number of poultry outbreaks for each epizootic. Horizontal lines indicate the day at which half of the poultry outbreaks have occurred (diamonds); error bars indicate 1 SD. E) Number of poultry outbreaks for each month for the 3 epizootics. HPAI, highly pathogenic avian influenza.

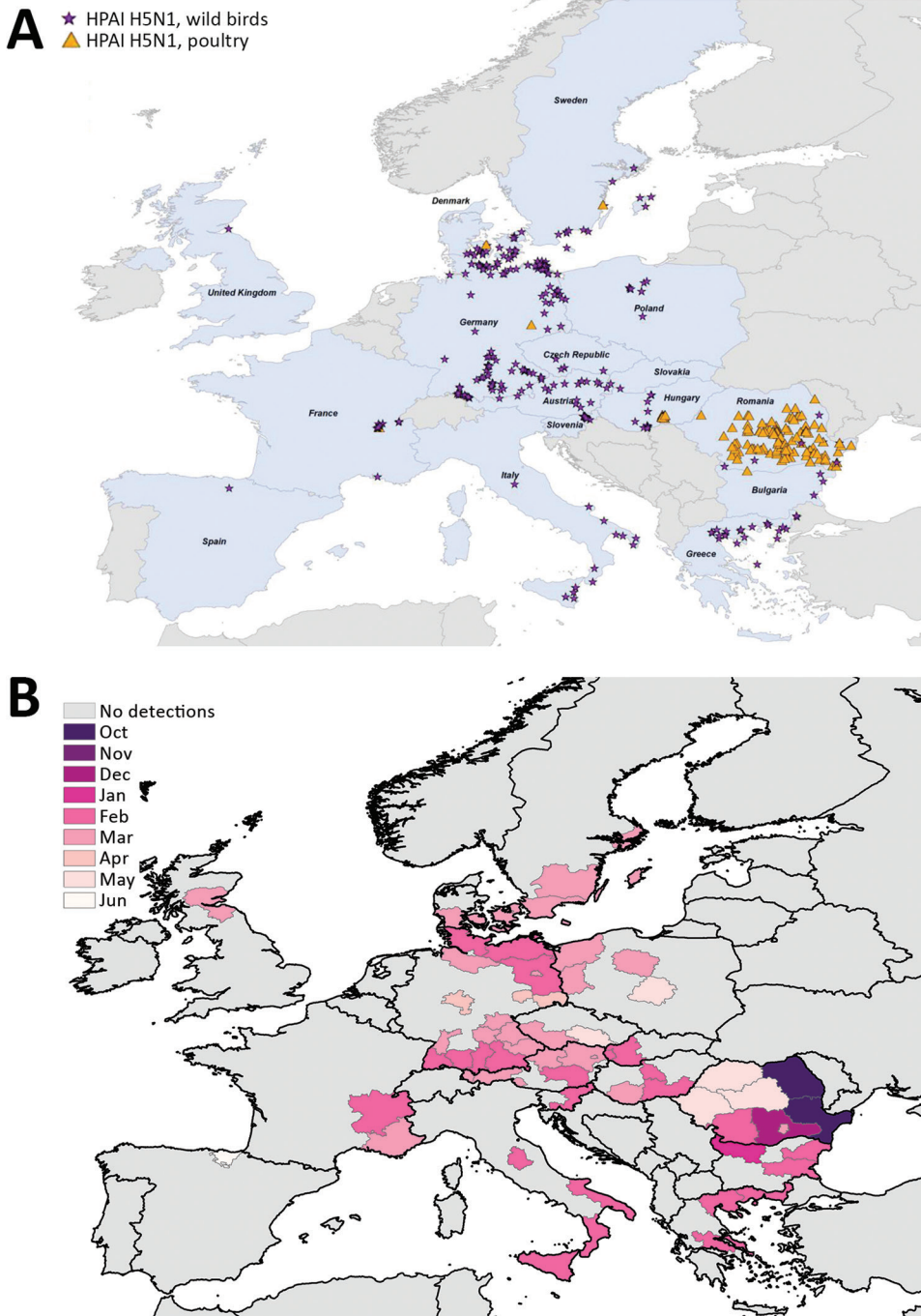
this dataset is generally less distinct and many sequences remain unresolved.

BEAST analyses (<http://tree.bio.ed.ac.uk/software/BEAST/>) also revealed that the 2014–15 epizootic viruses show the highest mean substitution rate (measured per site per year), followed by 2016–17 and then by the 2005–06 epizootic, which is significantly lower (one-way analysis of variance  $p < 0.001$ ) (online Technical Appendix Figure 6). These data are in agreement with the results of the root-to-tip regression analysis (online Technical Appendix Figure 7), which show a much steeper slope for the 2014–15 epizootic compared with the others. However, the spread of the data is high for the 2016–17 epizootic, where the SD of rates is an order of magnitude higher than that for the 2014–15 epizootic and 2 orders greater than for the

2005–06 outbreak. The nucleotide diversity for each epizootic (online Technical Appendix Figure 9) shows that per-site diversity (average pairwise nucleotide differences in a population) is lowest in the 2005–06 epizootic (0.0038), consistent with the lower substitution rate inferred from BEAST. The 2014–15 epizootic has the highest diversity (0.0086); the rate for 2016–17, calculated from viruses collected through June 2017, is 0.0063.

## Discussion

The 2016–17 epizootic of HPAI H5 clade 2.3.4.4 viruses in Europe has 5 times more outbreaks in poultry than observed in the H5 clade 2.2 epizootic in 2005–06 and 80 times more than in the H5 clade 2.3.4.4 epizootic in 2014–15. This study highlights the unprecedented



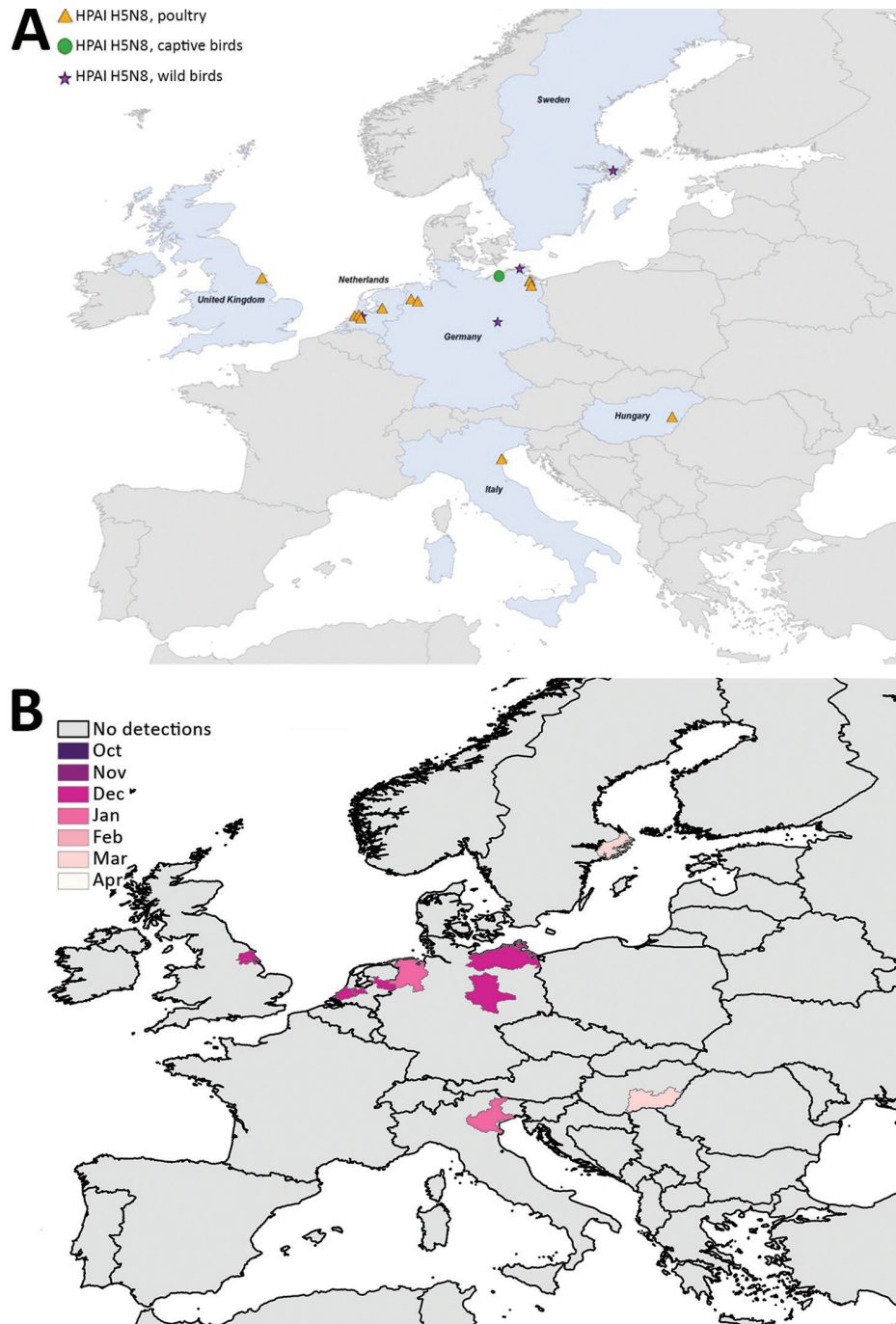
**Figure 3.** Geographic and temporal spread of the 2005–06 HPAI H5N1 epizootic. A) Location of each incident reported. Blue shading indicates countries where cases were reported. B) Month of first report of an HPAI H5N1 incident. Years given are epidemiologic years (October through September of the next year). HPAI, highly pathogenic avian influenza.

magnitude of the 2016–17 HPAI H5 epizootic in Europe, in terms of size (both number of poultry outbreaks and wild bird incidents), geographic spread, speed of incidents/outbreaks, and diversity of wild bird species reported infected. As a result, the economic impact is many times higher for 2016–17, which resulted in an  $\geq 8$ -fold increase in poultry that died or were culled.

A greater passive surveillance effort to detect influenza virus in wild birds was reported in the EU in 2006 than in

2016 (20,21). Despite reduced passive surveillance efforts in recent years, more virus detections were made in wild birds in calendar year 2016 compared with 2006, indicating a likely increase in viral burden within bird populations in Europe, leading to an increased risk for incursion into poultry. Although we found a lower rate of substitution and diversity in 2016–17 compared with 2014–15, the viruses in the 2016–17 epizootic might be more efficient in capacity to adapt and infect avian hosts. Different rates and diversity between



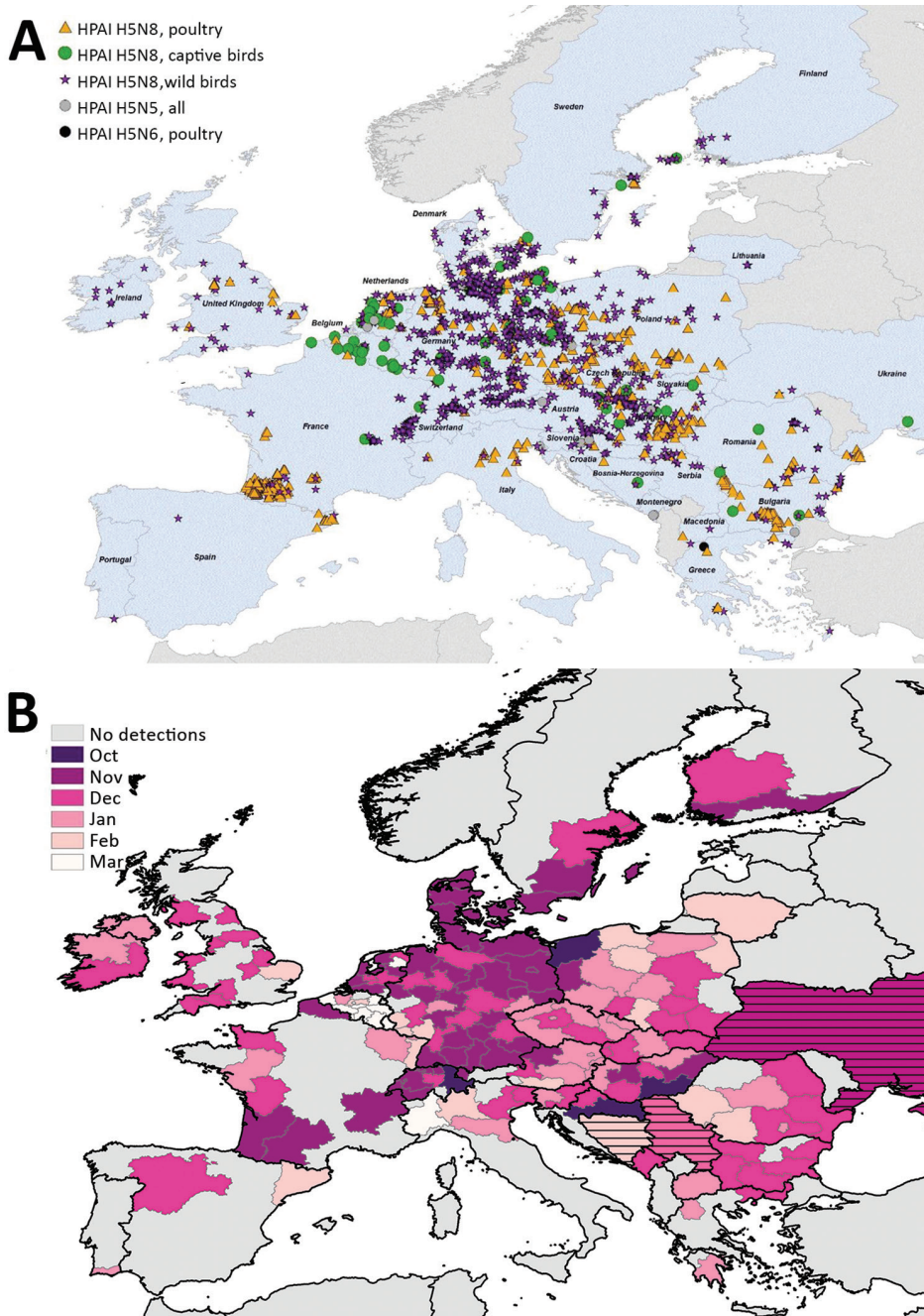


**Figure 4.** Geographic and temporal spread of the 2014–15 HPAI H5N8 epizootic. A) Location of each incident reported. Blue shading indicates countries where cases were reported. B) Month of first report of an HPAI H5N8 incident. Years given are epidemiologic years (October through September of the next year). HPAI, highly pathogenic avian influenza.

2005–06 and the 2 more recent epizootics may be caused by overall differences in the H5 lineages (clade 2.2 versus 2.3.4.4), which could influence viral spread. The greater genetic distances we observed in viruses detected in the 2014–15 epidemic could also be due to lower sensitivity of surveillance for this virus compared with the other 2 epidemics due to an apparently lower mortality rate in wild birds.

Extensive secondary spread is the most probable explanation for the large number of outbreaks reported in the

farmed duck sector in 2016–17, possibly because of rapid attenuation of viral symptoms. Hence, on several farms with clinically healthy birds, we detected HPAI infections through active epidemiologic tracings and not on the basis of clinical signs, as reported in data from some member states. The results may also indicate that infection and transmission between domestic ducks is relatively easy for these viruses. The type of husbandry practices and frequent movement of birds, coupled with poor biosecurity and lack

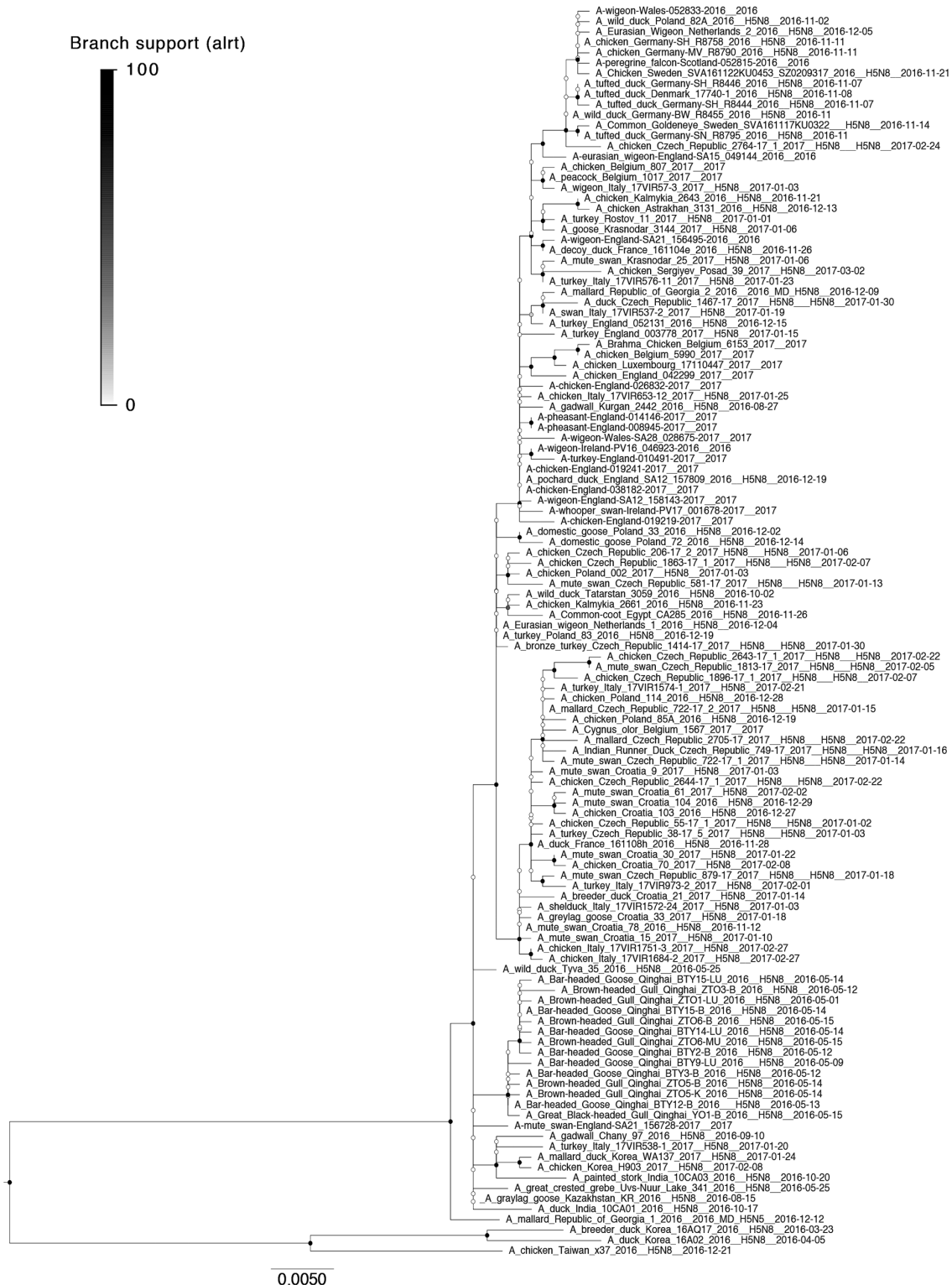


**Figure 5.** Geographic and temporal spread of the 2016–17 HPAI H5N8 epizootic. A) Location of each incident reported. Blue shading indicates countries where cases were reported. B) Month of first report of an HPAI H5N8 incident. Years given are epidemiologic years (October through September of the next year). HPAI, highly pathogenic avian influenza.

of robust hygiene practices, may also make the spread of the viruses between farms easier (22).

Swans and ducks were the predominant hosts infected in 2005–06 and 2016–17. Of interest, although mallards (*Anas platyrhynchos*) are the most frequently tested in EU passive surveillance (4), tufted ducks (*Aythya fuligula*) were the most commonly identified species of duck with HPAI in 2005–06 and 2016–17. In addition, the 2016–17 epizootic demonstrated a much expanded wild bird host range compared with previous outbreaks.

In light of these results, we recommend a review of the target species for avian influenza surveillance (5) to improve sensitivity of surveillance. Clarifying the precise origins of the current epizootic viruses from reported wild bird mortality data is problematic, because these data do not allow distinction between migratory carrier species and resident sentinel species. Many of the reported species are not migratory (e.g., mute swan or little grebes) and so might play a role as regional amplifiers of viruses but not in long-distance spread (23).



**Figure 6.** Maximum-likelihood tree from viral sequences of the 2016–17 highly pathogenic avian influenza H5 epizootic in Europe. Circles represent node support values, filled according to approximate likelihood ratio test (alrt) values 0–100. Light gray boxes indicate distinct clades with support >50 with isolates from Europe; dark gray boxes indicate clades with <50 or unresolved. Scale bar indicates nucleotide substitutions per site. An expanded figure showing trees for all 3 epizootic years is available online (<https://wwwnc.cdc.gov/EID/article/24/12/17-1860-F6.htm>).



Epidemic curves for the 3 epizootics were significantly different. The incidence values in order of magnitude were 2016–17 > 2005–06 > 2014–15. In the period of the review, the mean temporal distances to the midpoint in the poultry epizootic were different; 2014–15 was relatively short, consistent with the incursion into the poultry sector and potentially lower virus infectivity present in the wild bird reservoir, whereas in 2005–06 and in 2016–17, epidemic curves show a clear peak of detection of wild bird incidence preceding the peak of poultry incidences, which demonstrates the importance of wild bird surveillance.

For the 2016–17 epizootic, the epidemic curve shows a long extended tail with small sporadic peaks relating to localized but limited detection and spread in both poultry and wild birds (Figure 2, panel C). These data might suggest greater infection pressure from migratory birds in 2016–17, leading to higher risks for incursion, greater environmental contamination, and exposure of local indigenous wild bird populations and poultry. The observed spatiotemporal relationships between poultry incursions and wild bird detections represent a complex dynamic. Exploration of the epidemic curves by country in 2016–17 shows important differences that relate to the type of poultry production infected (online Technical Appendix Figure 7). For example, we detected infections in Hungary relatively early in the epizootic; their rapid peak and decline may reflect extensive infection within the major duck-producing regions and less susceptible populations through infection and depopulation. In contrast, infection in Germany and Poland was more consistent and may reflect a more continuous exposure and incursion risk into a variety of poultry sectors.

The viruses showed close genetic similarity to viruses contemporaneously circulating in Central and Southeast Asia. The lower genetic diversity observed in 2016–17 was accompanied by reassortment of all gene segments, as shown in previous studies (8,24,25). The high reassortment observed in the 2016–17 epizootic also resulted in novel NA reassortants such as the H5N6 and H5N5 viruses. The H5N6 viruses circulating in Europe were a reassortant of HPAI H5N8 and classical European LPAI present in wild birds (data not shown). We can clearly differentiate the genetic characteristics of this strain from viruses known to be circulating in poultry and wild birds in the Far East with occasional spillover to humans.

Epidemiologic results suggest 2 broad corridors of virus incursion in 2005–06 and 2016–17, through northern and central Europe with subsequent spread, later corroborated through phylogenetic analyses of the HA gene of the viruses from the 2016–17 epizootic. This dual incursion probably relates broadly to known postbreeding movements of northern duck species, which breed widely across northern Eurasia (11,13,26). These movements occur on a broad front, but ringing recoveries and other analyses

demonstrate movements from breeding areas from Siberia both southwest toward the Black and Aegean Seas and ultimately the coastal wetlands of the eastern Mediterranean, and further north and west through the Baltic Sea to coastal and other wetlands of the southern North Sea and northwestern countries (11–14). These represent migratory tendencies only; several studies have shown the high-level complexity of these movements and their variation due to both short-term weather patterns and longer-term climate change (27,28). The fact that these corridors were apparent in 2 temporally distant epizootics suggests the need for further research to focus surveillance in these areas.

This study presents many limitations (online Technical Appendix). Differences in the implementation of passive wild bird surveillance between countries, which are implied in the EU avian influenza annual report for 2016 (20), suggest that sensitivity of wild bird surveillance varies across countries (29), which could affect the distribution of cases we observed. The true probability of detecting HPAI is dependent on many factors that may influence both the frequency of wild bird deaths and the likelihood of identification and sampling of wild bird carcasses in different regions and countries. Public awareness, the current avian influenza status of the country area, media coverage, prevailing climatic conditions, available food sources, and removal by predators may affect wild bird mortality, detection rates, or both (30). Furthermore, the efficacy of passive surveillance is difficult to measure because capturing the expended effort depends on observation and testing of deceased birds. On the other hand, surveillance has high sensitivity in farmed poultry, mainly because of higher virulence and much closer observation of these populations.

Despite apparent heavy infection pressure in wild birds in 2016–17, the virus was not detected early in the epizootic in areas in eastern Europe, such as the Danube Delta, with high density of early migratory waterfowl. There were significant incursions in poultry in northern Europe, particularly Germany and Poland, and these areas also reported the greatest number of infected wild birds. This finding may reflect the implementation of enhanced surveillance in wild bird populations rather than true increased risk. Southwestern Europe had relatively few wild bird detections compared to the number of poultry outbreaks, perhaps because of the establishment of the virus in the duck production sector in southwestern France, not as a result of increased introductions from wild birds (31).

The extent of the 2016–17 H5 epizootic indicates an urgent need to reappraise the effectiveness of surveillance strategies in both wild and domestic birds and to monitor key populations for emergence of viral variants. The differences we observed in the 3 epizootics illustrate the difficulty of predicting HPAI epizootics. However, the temporal peak of wild bird detections preceding the peak of

poultry outbreaks at the EU level highlighted the utility of surveillance in wild birds, as observed in other studies (29). The spatial corridors of HPAI we identified may provide the basis for an increase in targeted surveillance to improve system sensitivity. Although the H5N8, H5N5, and H5N6 European-reassortant viruses have not been shown to infect humans and remain avian influenza-like strains with no evidence of key mammalian adaptation markers (27), their genetic volatility represents a potential threat that requires continuous monitoring and surveillance of virus incidence and genetics to continue to protect public safety.

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# Comparison of 2016–17 and Previous Epizootics of Highly Pathogenic Avian Influenza H5 Guangdong Lineage in Europe

## Technical Appendix

### Section A1. Materials and Methods

#### A1.1. Epidemiologic Analysis

Data were collected from the 3 major HPAI H5 epizootics in Europe: the HPAI H5N1 epizootic in 2005 and 2006 (“2005–06”); the HPAI H5N8 epizootic in 2014 and 2015 (“2014–15”); and the HPAI H5 epizootic in 2016 and 2017 (“2016–17”). For the 2016–17 epizootic, records were collected between October 19, 2016 and July 31, 2017. Epidemiologic data were obtained from the Animal Disease Notification System (ADNS), managed by the European Commission, DG SANTE (Directorate for Health and Food Safety), which is the official platform for EU MS to report outbreaks of notifiable animal diseases; and from country notifications sent to the EU Reference Laboratory for Avian Influenza (EURL – Animal and Plant Health Agency, Weybridge, UK).

The following data fields were collated: suspicion date, confirmation date, country, geographic coordinates, bird type (poultry, wild bird, or captive bird), bird species, number of birds at risk on the farm, total cases, total deaths, and total animals destroyed. Data available on the ADNS system may be subject to differences in interpretation from system users. Biases, data gaps, and uncertainty in the data uploaded to the ADNS system are listed in the section **Biases associated with ADNS data** in section A1.3 of this appendix.

All confirmed cases were identified from samples tested using recommended diagnostic tests (Commission Decision 2006/437/EC) as described in the OIE manual of diagnostic tests (EC 2006) (1).

All statistical analyses were performed in STATA 14 (StataCorp, College Station, TX, USA).

Epidemiologic analyses were done to:

- ***Describe the size of the epizootics:*** frequency of poultry outbreaks and wild bird incidents by country and poultry type or wild bird species were described.

- ***Investigate differences in geographic spread:*** spatial analyses were performed using ArcMap 10.2.2 (ESRI, USA) to visualize locations of poultry outbreaks and wild bird incidents, and to assess spatial progression of the epizootics by month. In addition, geographic spread was examined in relation to major wild bird migratory patterns. Countries were grouped into 4 regions based on the broad migration patterns of the major migratory water bird species affected by HPAI (Technical Appendix Figure 1). Justification for these groupings can be found under the heading **Derivation of geographic regions** in section A1.4 of this appendix. The regions were defined as follows:

- North of Europe: Belgium, Denmark, Estonia, Finland, Latvia, Lithuania, Luxembourg, Ireland, North France (above 45 latitude), North Germany (above 50 latitude), Norway, Poland, Sweden, The Netherlands, and United Kingdom.
- South-West Europe: Croatia, Malta, Italy, Portugal, Slovenia, South of France (below 45 latitude), and Spain.
- South-East Europe: Bulgaria, Cyprus, Greece, Hungary, Serbia and Romania.
- Central Europe: Austria, Czech Republic, Slovakia, South Germany (below 50 latitude), and Switzerland.

- ***Investigate differences in temporal spread:*** We compared the different epidemic curves. We tested for differences in the shape of epidemic curves using a 2-sample Kolmogorov–Smirnov test to measure the temporal distance to the median point of the poultry epizootic (day at which half of poultry outbreaks have occurred). In addition, seasonality was compared (period of the year where cases have occurred).

- ***Investigate differences in clinical presentation:*** Differences in the number of mass die-off events observed in wild bird species were tested as a measure of disease impact in wild birds.

Differences in clinical presentation in poultry were tested by comparing poultry morbidity and mortality data for each species in each epizootic. Estimation on morbidity using ADNS data was used from farms rearing only 1 species when possible (e.g., only ducks).

Morbidity was calculated based on 3 variables collected on ADNS:

- At risk = number of animals at risk of infection on the farm at time of investigation
- Cases = number of cases observed on the farm at time of investigation
- Deaths = number of bird dead on the farm at time of investigation

Due to different system users inputting data to the ADNS databank, it is suspected that while most observations in the “At risk” data field correspond to the total number of birds on the farm before the outbreak (deceased, moribund and healthy birds), in some instances this field may have been interpreted to represent the number of healthy animals remaining on the farm, or the total number of animals within an epidemiologic unit on the farm. For the “Cases” data field, it is suspected that some entries include both deceased and moribund birds within the estimation, while some entries only report moribund birds, alive with clinical signs. The following assumptions were made for the calculation of morbidity:

- Number of “At risk” was believed to be the total number of animals on the farm.
- The “Cases” field includes both the number of dead and moribund birds, except in those entries where the number of deaths is larger than number of cases (74 entries, which is 9.3% of poultry morbidity estimates). When the number of deaths is bigger than number of cases, the total number of cases was believed the sum of “cases” and “deaths.”
- When the “Cases” field was blank, and the number of “Deaths” was known, the number of deaths was used to represent the number of cases observed on the farm (n = 265, which is 33.4% of morbidity estimates).
- All farms with null “Cases” and “Deaths” were discarded, as these were considered to be farms without clinical disease, reported as dangerous contacts due to links with infected farms.

## A1.2. Phylogenetic Analysis

Virus haemagglutinin (HA) gene sequence data were obtained from countries' submissions to EURL; sequences generated as part of this study; and from the Global Initiative on Sharing All Influenza Data (GISAID) platform downloaded on June 2, 2017. To determine the genetic relationships among strains circulating in each epizootic, we analyzed viral sequence data from each outbreak separately. Haemagglutinin (HA) gene sequences of viruses from each epizootic were first subject to a quality control step where all duplicate sequences, sequences with <900 nt, and those with >10% undefined nucleotides (Ns) were discarded. Sequences in each dataset were aligned using MAFFT v7.305b, and trimmed to remove signal sequence at the N terminus and STOP codon at the C-terminus. Trimmed sequences were realigned using prank v.170427 with model parameters codon (empirical codon model) and F (force insertions to always be skipped).

IQ-TREE version 1.5.5 was used to infer maximum-likelihood trees from each dataset. The codon model was determined by the Model Finder algorithm within IQ-TREE, and both alrt (approximate likelihood ratio test, 1000 replicates) and bootstrap (100 replicates) were calculated to determine support for branching. The “best tree” was then annotated in FigTree v1.4.3.

Then, each dataset was down-sampled using CD-HIT-EST (Cluster Database at High Identity with Tolerance) to remove sequences with >99.9% sequence identity. The down-sampled datasets contained 244 (2005–6), 134 (2014–15) and 91 (2016–17) sequences.

Root-to-tip regression analyses were performed using Tempest v1.5 on the down-sampled datasets before Bayesian phylogenetic trees were inferred using BEAST v1.8.4 to determine the mean substitution rate and TMRCA (time to most recent common ancestor). The HKY site model was used with estimated base frequencies and gamma site heterogeneity with 4 gamma categories and 3 codon partitions. An uncorrelated relaxed lognormal clock was used with constant population prior and random starting tree. All priors were set to default except allMus which was set to a uniform distribution ranging from 0 to 1E100. MCMC was set to 100 million generations. Log files were analyzed in Tracer v1.6.0, to determine convergence, and check ESS values were beyond threshold (>200). Tree annotator v1.8.4 was used to generate a maximum credibility tree (MCC) using 10% burnin and median node heights. The MCC tree was



then annotated to include time scale and mean substitution rate in FigTree v 1.4.3. The nucleotide diversity for each outbreak was measured using the PopGenome package in R.

### **A1.3. Biases Associated with ADNS Data**

**The following data gaps and uncertainties were detected in the poultry data:**

*Biases related to notification date:* Date of suspicion was missing for 24 outbreaks in 2005–06 and 319 outbreaks in the 2016–17 epizootics. Date of confirmation was used as the date of suspicion for the epidemic curves. It is possible that some countries have used the same confirmation date to report all incidents that have occurred within a week. This is possible due to the high work load pressure during the period with large number of outbreaks.

*Biases related to morbidity and mortality estimates:* Assumptions and uncertainties of the data have been described in section A1.1 of this online appendix. Number of cases and deaths were missing for many of the poultry outbreaks reported, which reduced the power of the analysis to detect differences. Information on cases were reported for 530 outbreaks (38% of all poultry outbreaks; 26% of outbreaks in 2005–06; 85% of outbreaks in 2014–15; and 40% of outbreaks in 2016–17). Information on number of deaths were reported for 776 outbreaks (56% of all poultry outbreaks; 86% of outbreaks in 2005–06; 62% of outbreaks in 2014–15; and 50% of outbreaks in 2016–17). When possible morbidity was estimated for farms having unique species, however this was not possible in all cases (e.g., Ducks in 2005–06). Morbidity and mortality estimates may be biased in some cases due to the existence of other poultry types on the premises (more or less susceptible to HPAI).

*Biases related to flock size:* Most observations in the “At risk” category should indicate number of birds reported on the farm, however it may be possible that some entries indicate only number of birds in one of the buildings of the farm.

*Bias related to differences between countries:* Variation in the poultry demographics between countries, regions within countries and over time may influence the detection of AI. Some of the variables implicated in this include: industry structure (high levels of organization and/or industry veterinarians may effect detection rates), industry engagement (aware of AI and proactive in reporting disease symptoms– or not), density of poultry systems (poultry farms clustered in close proximity), poultry types (anseriforme poultry types less likely to present

disease symptoms for detection), type of poultry production systems (indoor/outdoor/back yard flocks), variation in sampling strategies between countries and over time may lead to misrepresentation of the true distribution of AI poultry outbreaks.

Bias could also be introduced in the following ways: sensitivity of passive surveillance (procedures for detection by state vet services), intensity of scanning surveillance (serology), frequency of serology positive PCR follow-ups (e.g., sampling at slaughter influence proportion of PCR follow-ups?), and changes to intensity of surveillance following recent outbreaks in close proximity. Differences on implementation of passive surveillance in wild birds by countries are shown in the EU Avian Influenza surveillance report (2).

**The following data gaps and uncertainties were detected in the wild bird ADNS data:**

*Biases related to “Notification date”:* “Date of suspicion” was missing for 1,191 wild birds incidents (76.4%) in the 2016–17 epizootics and date of confirmation was used as the date of suspicion for the epidemic curves. In addition, seven wild bird incidents had the same suspicion and confirmation date. It is possible that some countries have used the same confirmation date to report all incidents that have occurred within a week. This is possible due to work load pressure during the period with high number of outbreaks.

*Biases related to number of wild birds death reported by incident:* It is possible that for many incidents countries reported only those animals that they have tested and confirmed positive and did not report all the birds found dead in each incident.

*Heterogeneity in wild bird species and density across space and time may lead to differences in detection rates in some of the following ways:* density of wild birds combined with level of human traffic influence the likelihood of an individual coming across a sick/dead bird and reporting it, species of wild birds are heterogeneously distributed across Europe, some species are less likely to exhibit disease symptoms/suffer mortality, temporal variation in wild birds densities and species types due to seasonal migration.

Wild bird surveillance is carried out actively (sampling live healthy birds, mainly detecting LPAI H5 and H7) and passively (sampling sick or found-dead birds, mainly detecting HPAI). Each of these strategies may introduce bias in the following ways:

- Active surveillance of live healthy birds: There are differences in time and resources countries dedicate to the capture of live birds to sample. This influences the likelihood of finding infected birds, if present. This may be one reason for the high detection rate of infected wild birds in Germany. Active surveillance only samples birds in the target species list.
- □Passive surveillance of sick/injured and birds found dead: Variety in state effort put into searching for dead wild birds to sample. Public awareness in reporting dead wild birds may be different between countries, Likelihood of detection of wild birds found dead may vary with species (e.g., big white birds easier to spot than little brown birds. Little birds scavenged more readily), Increase in local surveillance spatially and temporally during an outbreak

#### **A1.4. Derivation of Geographic Regions**

Each migratory waterbird species has a migration pathway that comprises a unique spatial-temporal combination of habitats used and geographic areas occupied. While typically there is high year-to-year consistency in migration patterns, many species show flexibility in occurrence related to the short-term influences of weather, as well as longer term consequences of changing climate.

Migration pathways for species of similar ecology have been aggregated into flyways – defined as: “A flyway is the entire range of a migratory bird species (or groups of related species or distinct populations of a single species) through which it moves on an annual basis from the breeding grounds to non-breeding areas, including intermediate resting and feeding places as well as the area within which the birds migrate” (3).

There is good knowledge of the flyways and areas of occupancy of most European waterbirds, summarized both in published atlases, for ducks, geese and swans (4) and waders (5), as well as online for other species (e.g., Wings over Wetlands).

To summarize geographically the broad occurrence of H5 virus outbreaks for this study in the context of migratory waterbird distribution, we assigned countries to one of 4 regions based on the combination of broad distribution and migration patterns of relevant duck, goose

and swan species (although informed by the distribution of other migratory waterbirds as relevant).

Movements of many ducks are similar, showing post-breeding movements from tundra and taiga habitats in western and central Russia, westwards to non-breeding areas in western and southern Europe (4,6,7). Recoveries of ringed birds and more recent new tracking technologies indicate population structuring: broadly, with two separate biogeographical populations occurring i) in northwest Europe (UK and Ireland, northern France, and countries surrounding the North and Baltic Seas); and ii) in the countries of the Mediterranean basin from Iberia to Israel, together with the Black Sea. This is the case for Eurasian Wigeon (*Anas penelope*), Gadwall (*Anas strepera*), Common Teal (*Anas crecca*), Common Pochard (*Aythya ferina*), and Tufted Duck (*Aythya fuligula*). For some species such as Mallard (*Anas platyrhynchos*), the southern wintering Mediterranean birds are split into western and eastern Mediterranean populations, while the population range of southern wintering Pintail (*Anas acuta*) and Northern Shoveler (*Anas clypeata*) also extends to west and sub-Saharan Africa. Other species, such as Greater Scaup (*Aythya marila*), other seaducks, and arctic-breeding geese occur around the North Sea basin without more southerly Mediterranean wintering areas.

The 4 regions defined in this paper (North, South-West, South-East, and Central Europe) aim to broadly categorize distinct geographic areas used by different biogeographic populations of waterbirds. For ease of data-handling, whole countries are assigned to different regions other than for France and Germany. Waterbirds using the coasts and inland wetlands of southern France were assigned to South-West Europe, while southern Germany was included in Central Europe.

## **Section A.2 – Comparative Statistical Analysis and Results**

### **A2.1. Number of wild bird deaths reported by incident in the 3 epizootics**

Results show that number of wild birds death reported by incident were different between the 2005–06 and 2016–17 epizootic ( $p < 0.001$ ) (Technical Appendix Figure 2).

### **A2.2. Distribution of poultry flock sizes of infected poultry premises between epizootics**

Technical Appendix Figure 3 shows the distribution of poultry flock size in farms detected with H5 HPAI in the 3 epizootics.

### **A2.3. Frequency of poultry outbreaks and wild bird incidents by European region between the 3 epizootics**

During the H5N1 HPAI outbreak of 2005–06 the majority of poultry detections were made in the South-East European region (98%), while detections were seen across all regions of Europe in the H5N8 HPAI outbreak 2016–17, with the majority of detections reported from South-East Europe and South-West Europe (Technical Appendix Figure 4). Bosnia and Herzegovina ( $n = 1$ ), Serbia ( $n = 4$ ), FYRO Macedonia ( $n = 1$ ) and Ukraine ( $n = 2$ ) were not included in the regions. The Pearson Chi-square test to compare the epizootics indicates that distribution of poultry outbreaks in the four regions does differ between epizootics ( $p < 0.001$ ). Pearson Chi-square test indicate that distribution of wild birds incidents report in the 4 regions is also different between 2016–17 and 2005–06 epizootics ( $p < 0.001$ ), but not significantly different with 2016–17 and 2014–15 ( $p = 0.282$ ) and only weak evidence of difference between 2005–06 and 2014–15 ( $p = 0.05$ ).

### **A2.4. Comparison of the epidemic curves between the 3 epizootics**

Three types of analysis were done to assess differences in the distribution and values of the epidemic curve; differences in the distance to the day at which half of poultry outbreaks have occurred; and difference in seasonality.

Differences in the distribution of the 3 poultry epidemic curves

A 2-sample Kolmogorov–Smirnov test to assess equality of distribution functions was performed. Results indicate that the 2016–17 epizootic contains significantly higher values than the 2005–06 ( $p < 0.001$ ) and 2014–15 ( $p < 0.001$ ) epizootics, and that the 2005–06 epizootic contains significantly higher values than the 2014–15 epizootic ( $p\text{-value} < 0.001$ ).

Differences in distance to the day at which half of poultry outbreaks have occurred

The distance (“mean distance”) was calculated using day 1 as the day when the virus was first detected in Europe, either in wild birds or poultry. Results indicate that half of poultry outbreaks were reported on day 198 in 2005–06 epizootic, on day 38 in the 2014–15 epizootic and on day 91 in the 2016–17 epizootic. T-test and Wilcoxon rank sum test were performed to assess for evidence of differences between mean distances of 2 epizootics. The results of the analysis show that distance were significantly different between the 3 epizootics.



#### Difference in seasonality of poultry outbreaks between epizootics

The frequency of poultry outbreaks per month were examined. Pearson Chi-square tests were performed to assess for statistical difference in the distribution of poultry outbreaks per month, using pairwise comparison between epizootics. Results indicate that the distribution are significantly different between the three outbreaks ( $p$ -value<0.001).

#### Epidemic curves in the most important countries in the 2016–17 HPAI H5N8 epizootic

Epidemic curve of the 2016–17 HPAI H5N8 epizootic in Europe for the 6 countries most affected by the epizootic are shown in Technical Appendix Figure 9.

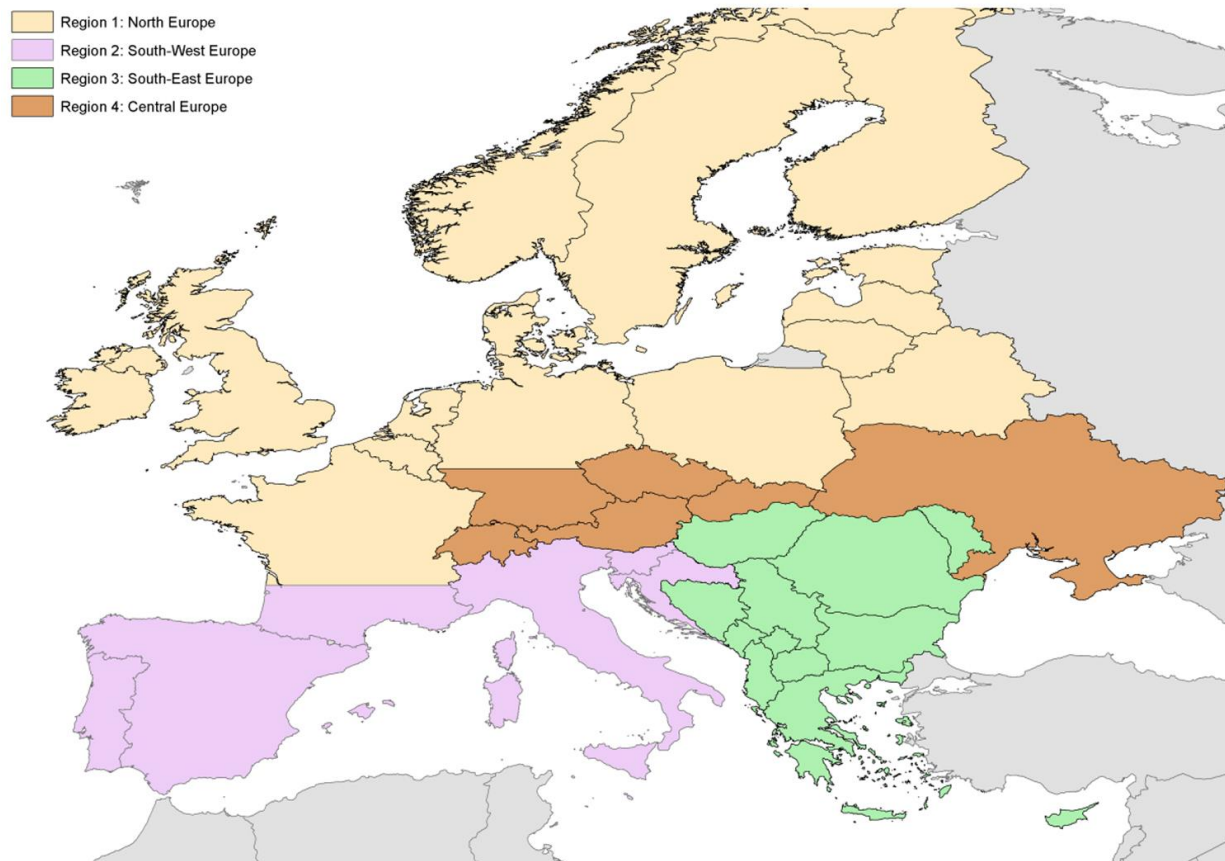
### **Section A4. Additional Results Phylogenetic Analysis**

Technical Appendix Figures 6–8 show additional results of the phylogenetic analysis conducted.

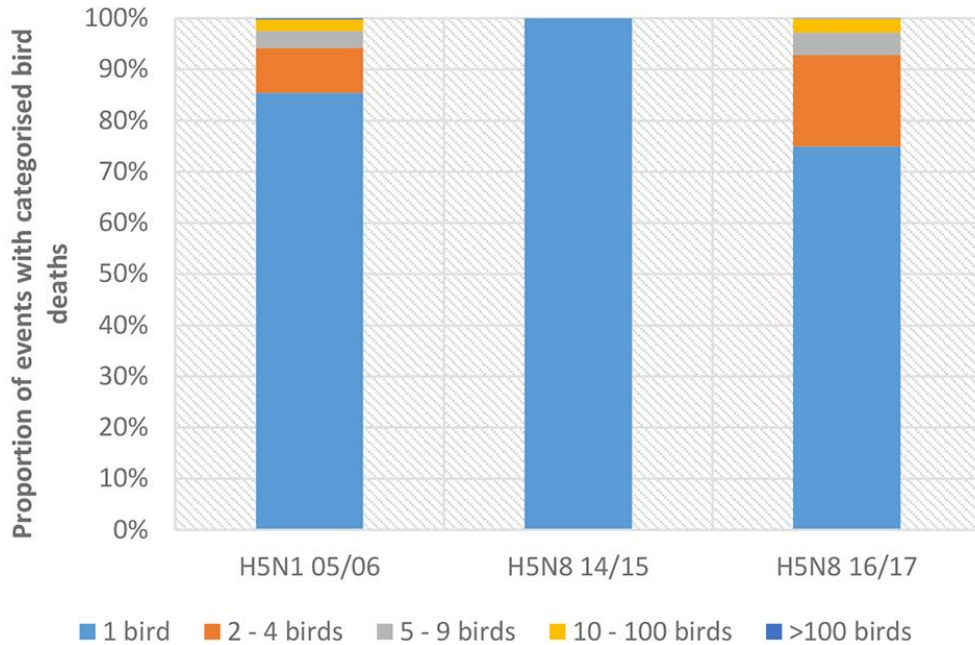
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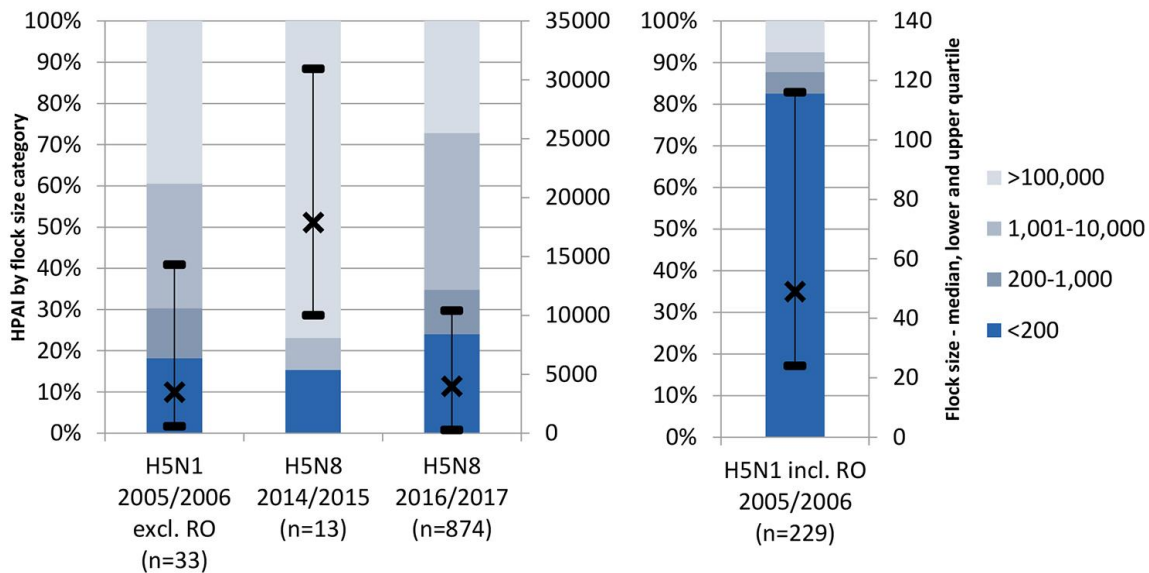
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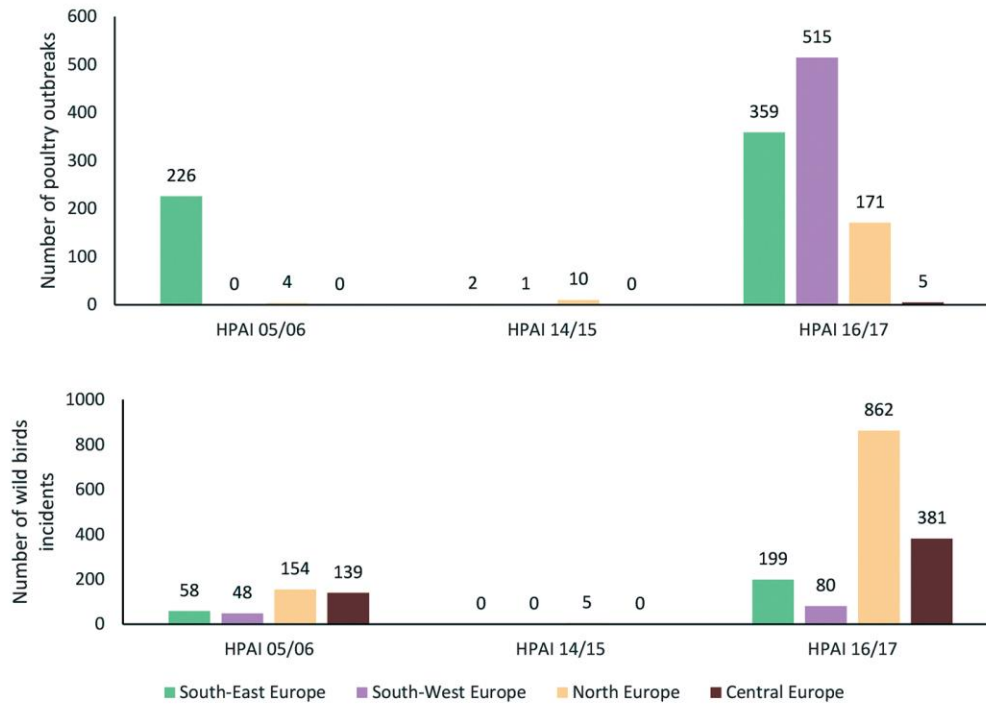
**Technical Appendix Figure 1.** Map of European regions based on wild bird migratory patterns, used for analysis of highly pathogenic avian influenza detections.



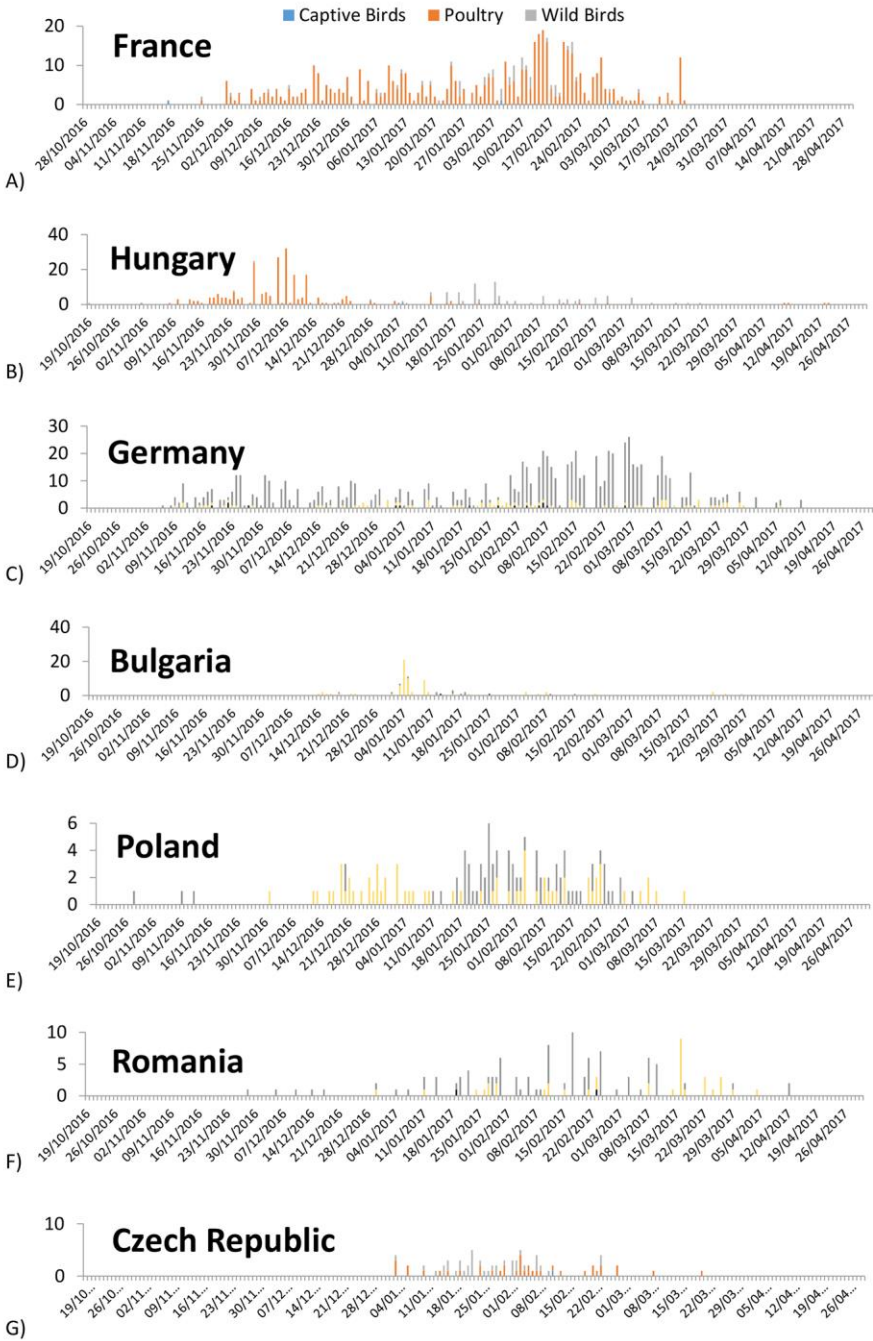
**Technical Appendix Figure 2.** Distribution of wild birds' incidents per category of number of deaths reported in the 3 epizootics.



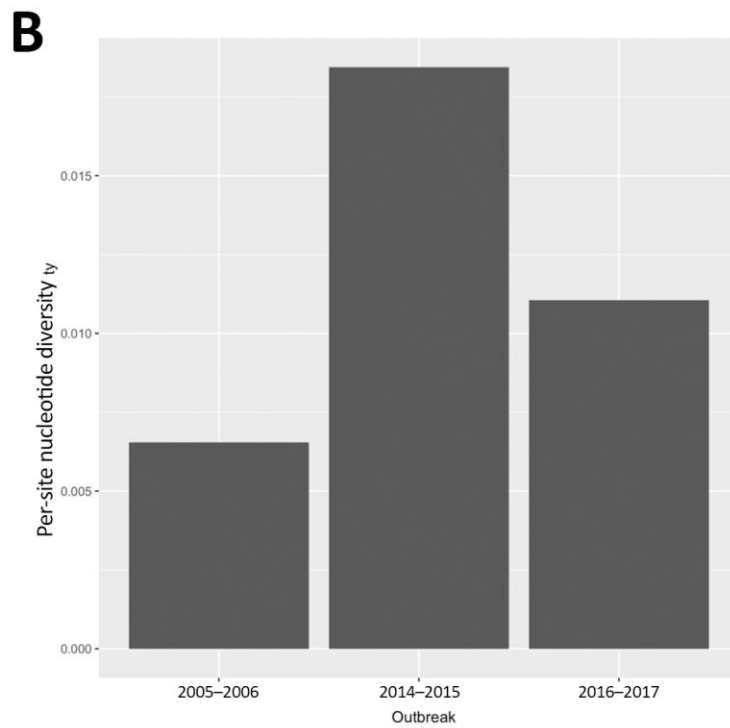
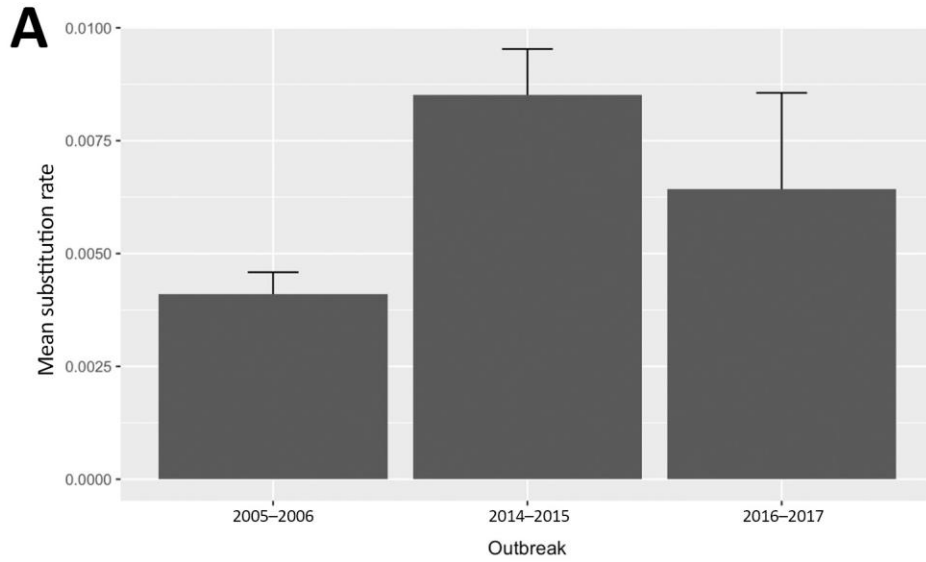
**Technical Appendix Figure 3.** Distribution of poultry flock sizes with a detection of H5 highly pathogenic avian influenza in 3 European epizootics. Crosses represent the median flock size and the interquartile intervals. RO, Romania.



**Technical Appendix Figure 4.** Number of poultry outbreaks (top) and wild bird incidents (bottom) reported in four European regions.

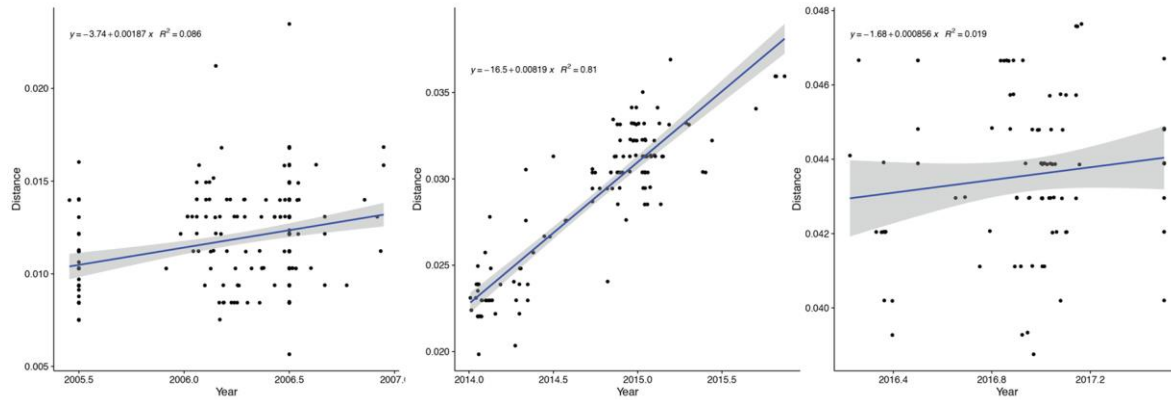


**Technical Appendix Figure 5.** Epidemic curve of the 2016–17 HPAI H5N8 epizootic in Europe (until April 30, 2017): A) France; B) Hungary; C) Germany; D) Bulgaria; E) Poland; F) Romania; G) Czech Republic.

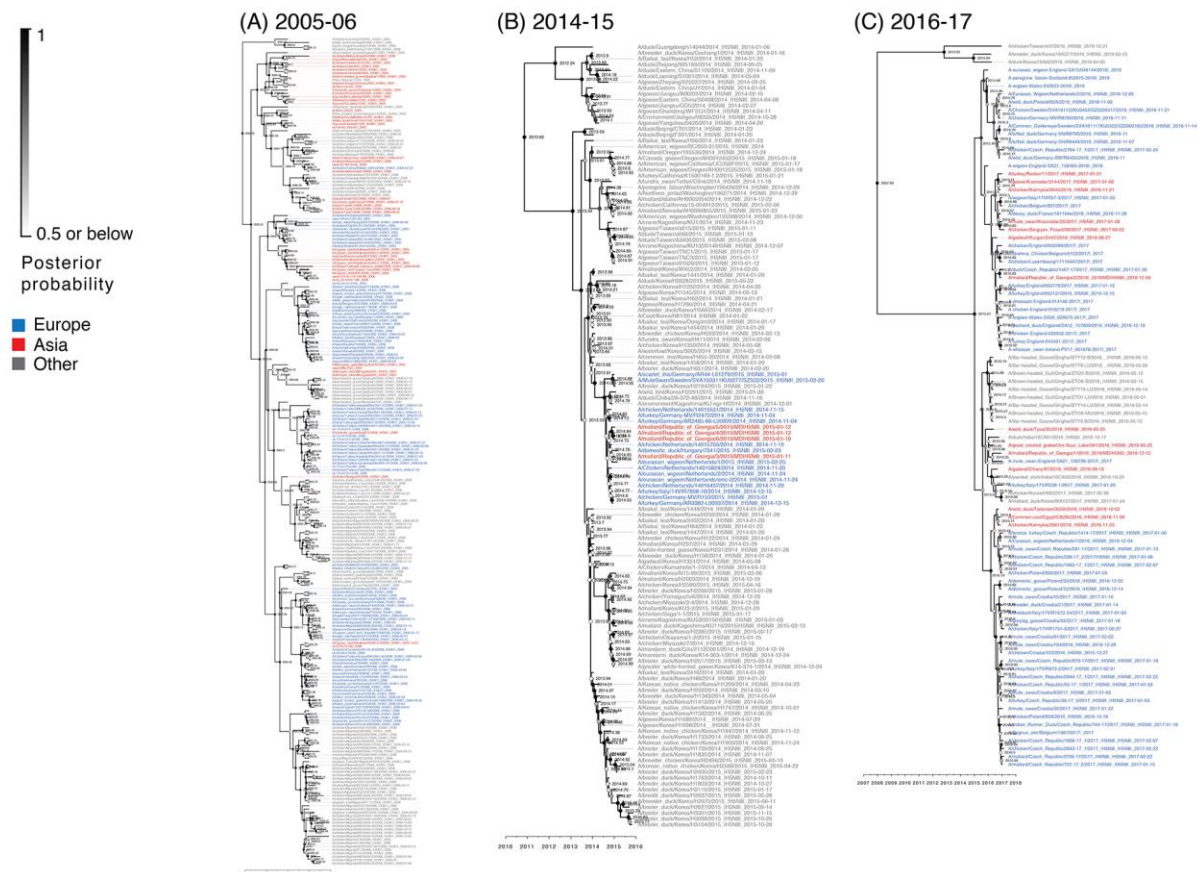


**Technical Appendix Figure 6.** A) Comparison of mean substitution rates (measured per site per year, with standard deviation) of H5 viruses in 2005–06, 2014–15, and 2016–17 outbreaks. B) Comparison of per-site nucleotide diversity, defined as average number of nucleotide differences per site between 2 DNA sequences in all possible pairs, in the sample population for viral population HA for each outbreak.

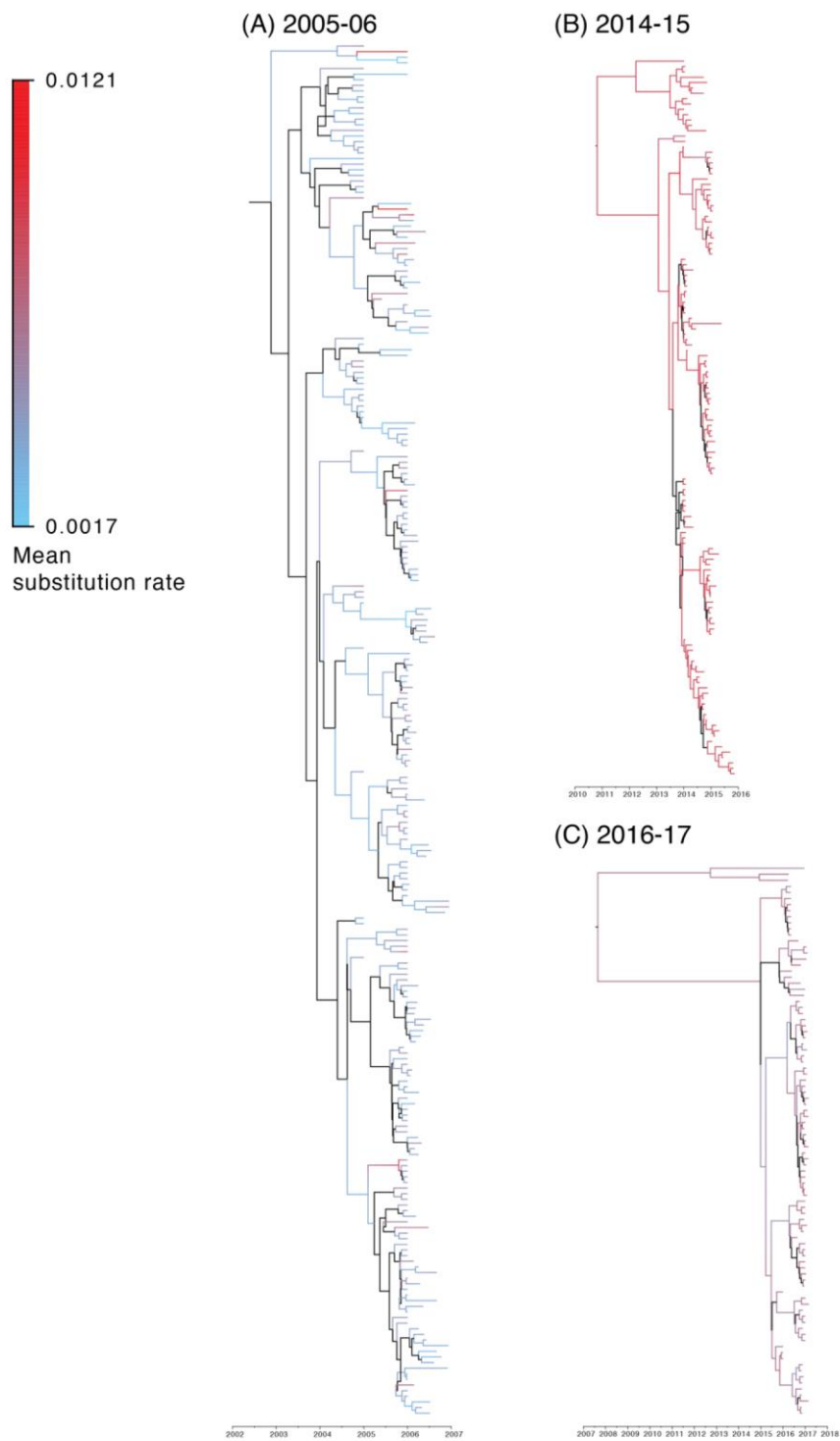




**Technical Appendix Figure 7.** Root to tip regression for ML trees generated from 2005–06, 2014–15, and 2016–17 outbreak viruses.



**Technical Appendix Figure 8.** BEAST MCC trees from viral sequences from 2005–06, 2014–15, and 2016–17 outbreaks of highly pathogenic avian influenza in Europe. Circles represent node support values, filled according to the posterior probability values ranging from 0–1. Clades with European isolates are highlighted in gray. In the 2016–17 tree, black arrows represent unresolved European sequences.



**Technical Appendix Figure 9.** BEAST MCC trees from downsampled viral sequences from 2005–06, 2014–15, and 2016–17 outbreaks of highly pathogenic avian influenza in Europe. Minimum and maximum rates are normalized across the 3 trees to enable comparison of rates.