

Highly Pathogenic Avian Influenza A(H5N8) Virus Spread by Short- and Long-Range Transmission, France, 2016–17

François-Xavier Briand, Eric Niqueux, Audrey Schmitz, Claire Martenot, Martine Cherbonnel, Pascale Massin, Florian Kerbrat, Marina Chatel, Carole Guillemoto, Cecile Guillou-Cloarec, Katell Ogor, Aurélie Le Prioux, Chantal Allée, Véronique Beven, Edouard Hirchaud, Yannick Blanchard, Axelle Scoizec, Sophie Le Bouquin, Nicolas Etteradossi, Béatrice Grasland

We detected 3 genotypes of highly pathogenic avian influenza A(H5N8) virus in France during winter 2016–17. Genotype A viruses caused dramatic economic losses in the domestic duck farm industry in southwestern France. Our phylogenetic analysis suggests that genotype A viruses formed 5 distinct geographic clusters in southwestern France. In some clusters, local secondary transmission might have been started by a single introduction. The intensity of the viral spread seems to correspond to the density of duck holdings in each production area. To avoid the introduction of disease into an unaffected area, it is crucial that authorities limit the movements of potentially infected birds.

Influenza A viruses are enveloped viruses of the *Alphainfluenzavirus* genus in the *Orthomyxoviridae* family. Their negative-stranded RNA genome consists of 8 segments encoding a total of 10–14 proteins. Avian influenza viruses (AIVs) are classified on the basis of antigenic differences in their surface glycoproteins, hemagglutinin (H1–H16) and neuraminidase (N1–N9) (1). H5 and H7 subtypes can become highly pathogenic avian influenza (HPAI) viruses after the evolution of multiple basic amino acids in the cleavage site of hemagglutinin protein (2,3). This mutation enables the virus to replicate efficiently in all organs, causing a severe and often fatal systemic disease. In contrast, the cleavage site of hemagglutinin in low pathogenicity AIVs lacks these multiple amino acids, restricting viral replication to the respiratory and digestive tracts. Low pathogenicity AIVs

cause subclinical or mild disease that can be aggravated by secondary infections (4,5). Because H5 and H7 AIVs can evolve to be highly pathogenic, the diseases caused by these subtypes are notifiable to national and international bodies (6).

Since 1996, highly pathogenic H5 viruses of the A/goose/Guangdong/1/96 (Gs/GD/96) lineage have caused recurrent outbreaks with high death rates in birds. These HPAIs are categorized into 10 distinct clades (0–9) on the basis of hemagglutinin sequences (7). These clades are found in Asia; a few have spread to Africa, Europe, and North America (8–10). Europe experienced major introductions of H5N1 of clade 2.2 during 2005–2007 and H5N8 of clade 2.3.4.4 during 2014–2020 (11–14). Many reassortments were observed on Gs/Gd/1/96-like viruses, especially within clade 2.3.4.4. The reassortments generated several subtypes including H5N1, H5N2, H5N5, H5N6, and H5N8 (11,15–17). During winter 2016–17, twenty-nine countries in Europe reported 1,576 cases of Gs/Gd/1/96-like H5N8 infections in wild birds and 1,134 in poultry, especially domestic ducks (18).

During this outbreak, researchers identified 6 HPAI A(H5N8) genotypes in Europe; 2 of these genotypes were identified using 6 sequences from infected birds in France (19). France had 539 cases of HPAI A(H5N8) infections, 51 in wild birds and 488 in poultry flocks, most of which occurred at duck farms producing foie gras (18). A previous study used spatiotemporal analysis of clinical cases comprising 2 distinct epizootic periods in southwestern France (20). The first period spanned November 28, 2016–February 2, 2017 and comprised 4 spatiotemporal clusters (20). The second period spanned February 3–March 23, 2017 and comprised a single spatiotemporal cluster (20).

Author affiliation: Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, Ploufragan, France

DOI: <https://doi.org/10.3201/eid2702.202920>

During the first period, the disease spread mainly among local farms; during the second period, after local farm-to-farm spread, the average distance between affected farms increased (20). To limit viral spread among poultry farms, the French Ministry of Agriculture and Food established protection zones (3 km radius) and surveillance zones (1 km radius) around outbreak sites according to European Union regulations (21). Additional control measures included preventive culling of poultry inside surveillance zones and of outdoor palmipeds inside protection zones (21). We sequenced 212 whole genomes of HPAI A(H5N8) viruses infecting wild and domestic birds during the outbreak in France. We used these molecular data to identify the geographic distribution and track the spread of H5N8 genotypes.

Material and Methods

Sampling

We collected oropharyngeal and cloacal swab samples from wild birds that had died of suspected H5N8 infection and from domestic or captive birds that had clinical signs of avian influenza. Official veterinarians from the Ministry of Agriculture and Food collected samples from poultry in surveillance zones before they were transferred or culled (21). Staff at district laboratories approved by the Ministry of Agriculture and Food suspended the swab samples in 2 mL of phosphate-buffered saline (PBS) and separated samples from domestic poultry into 5 pools.

Detection and Characterization of HPAI A(H5N8) Genomes

Staff at the district laboratories extracted viral RNA from each pool using the RNeasy Mini Kit (QIAGEN, <https://www.qiagen.com>) according to the manufacturer's instructions. They tested RNA samples by real-time reverse transcription PCR selective for the matrix gene and H5 gene; pathotype was determined as described (22) at the French National Reference Laboratory for Avian Influenza (Ploufragan, France). Samples from domestic poultry that had a cycle threshold (C_t) value <30 underwent whole-genome sequencing at the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (Ploufragan). All AIV-positive samples from wild birds, regardless of C_t value, also underwent whole-genome sequencing at the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail. We amplified viral genomes with real-time reverse transcription PCR using specific primers at the 5' and 3' conserved ends of all 8 AIV genome segments

(23). We sequenced amplicons with Ion Torrent technology (ThermoFisher Scientific, <https://www.thermofisher.com>). Libraries were prepared by using the Ion Xpress Plus Fragment Library Kit (ThermoFisher Scientific), selected by size, and cleaned by using the Agencourt AMPure XP (Beckman Coulter Life Sciences, <https://www.beckman.com>). We conducted emulsion PCR on the Ion OneTouch 2 system and subsequent enrichment of template particles on the Ion OneTouch ES system using the Ion PI template OT2 200 Kit version 3 (ThermoFisher Scientific). We loaded the samples onto a PI chip and sequenced them on an Ion Torrent Proton (ThermoFisher Scientific). We obtained the consensus sequence by comparing the de novo analysis with reference sequences from the Influenza Research Database (<https://www.fludb.org>) (24). We downsampled the reads to fit a coverage of 80× and submitted them to the SPAdes version 3.1.1 de novo assembler (<http://cab.spbu.ru/software/spades>). We submitted the de novo contigs to BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) on a local nucleotide database. For each segment, we selected the best matches for Bowtie 2 alignment (25). Finally, we compared de novo assemblies and alignment on the references and assessed their strict identities. We retained only the sequences with a coverage of $\geq 30\times$ for all segments for further analysis. For the following analyses we considered only sequences from nucleotide positions 20–2248 for polymerase basic (PB) 2 protein, 4–2259 for PB 1 protein, 41–2151 for polymerase acidic (PA) protein, 49–1704 for hemagglutinin, 14–1458 for nucleoprotein (NP), 50–1385 for neuraminidase, 38–936 for matrix protein, and 28–815 for nonstructural protein, according to the first ATG. We submitted sequences to GenBank (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/27/2/20-2920-App1.pdf>).

Phylogenetic Analysis

For the phylogenetic analysis, we used only samples with complete sequences for each segment. We aligned the sequences with ClustalW (<http://www.clustal.org>). We used MEGA version 7.0 software (26) to construct maximum-likelihood phylogenetic trees with 500 bootstrap replicates using the Tamura 3-parameter model. Then, we compared each segment that was representative of a phylogenetic group (i.e., closed sequences with >98% nucleotide identity) to sequences available in the GISAID database (<https://www.gisaid.org>).

For each sequence, we concatenated 8 AIV gene segments and tested them for reassortment using the RDP4 software (27) with the SIScan, Bootscan, RDP, MaxChi, and GENECOV methods. We estimated the

time to most recent common ancestor (tMRCA) of the viral sequences by performing Bayesian coalescent phylogenetic analyses in BEAST version 1.7 (28). The models considered constant size, exponential growth, expansion growth, and Bayesian Skygrid for coalescent model in combination with a strict or uncorrelated lognormal clock model. We chose the best model on the basis of Akaike's Information Criterion value (29). We applied the uncorrelated lognormal molecular clock with the SDR06 model of nucleotide substitution and Bayesian Skygrid coalescent model (30) as in previous studies (8,19). We ran the model for 40 million generations with sampling evolutionary parameters every 4,000 generations. We visualized the trace files with Tracer 1.6 (<http://beast.community/tracer>) to check that the effective sample size values were >200, which corresponded to an acceptable number of independent samples (31). After removing a 10% burn-in with TreeAnnotator version 1.7.5 (<https://beast.community/treeannotator>), we generated maximum clade credibility trees. We annotated the trees with Figtree version 1.4 (<http://tree.bio.ed.ac.uk/software/figtree>). We visualized the

evolution of the effective population size of A(H5N8) viruses in southwestern France using Icytree (32).

Potential Transmission Networks

We reconstructed the potential transmission networks using a minimum spanning tree from PopART version 1.7 (33) corresponding to a parsimony method to reconstruct the relationships among highly similar genomes. We analyzed 197 genomes of H5N8 viruses from southwestern France and determined the number of local geographic clusters by testing the model using 2–8 clusters; 5 geographic clusters produced the most consistent relationship between geographic clustering and genome similarity.

Results

Epizootic Case Situation

During winter 2016–17, France declared 539 cases of HPAI H5 infection, the second-highest number of cases in Europe. In total, 488 cases were in domestic or captive birds, primarily ducks, and 51 cases were in wild birds (Figure 1). The 488 domestic cases were

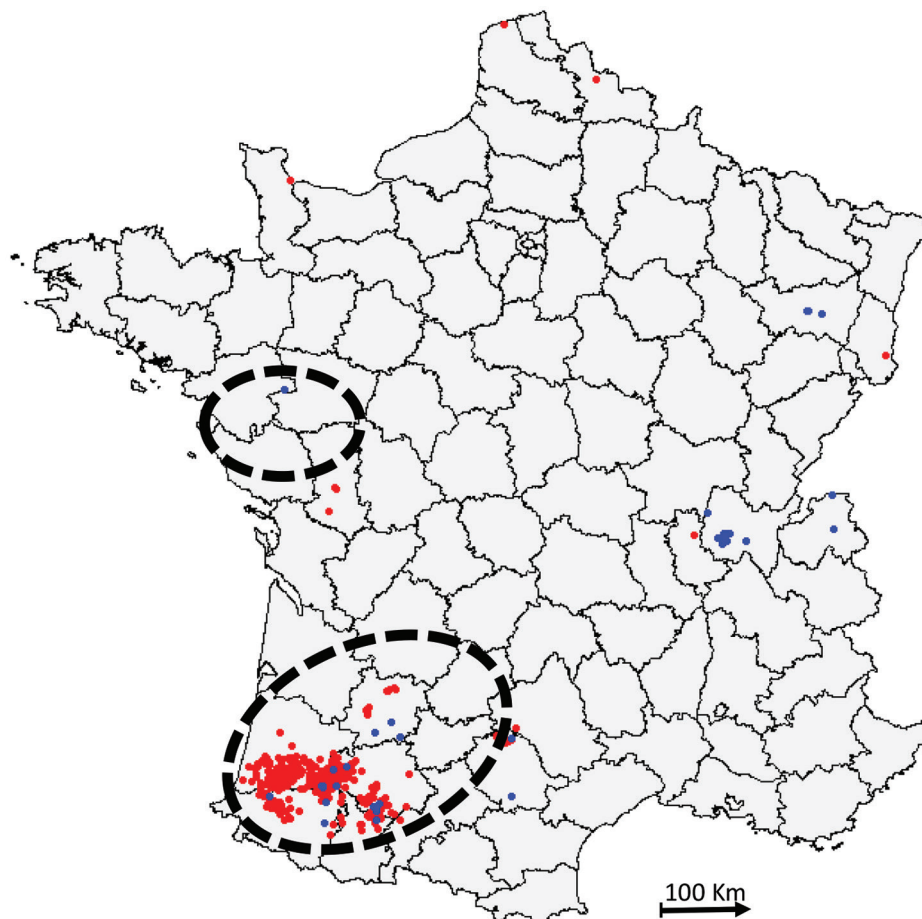


Figure 1. Distribution of highly pathogenic avian influenza H5N8 cases, France, 2016–17 (database of the French National Reference Laboratory for Avian Influenza). Blue indicates cases in wild birds; red indicates cases in domestic or captive birds. Dashed circles indicate zones of high duck farm density (34).

mainly in southwestern France, whereas H5N8 infection was more common in wild birds in other areas of France (Appendix Table 1). Seventeen cases were detected in wild birds, mostly common buzzards, in southwestern France, whereas cases in wild birds from other areas were in waterfowl (mostly swans). During this period in southwestern France, other AIVs were also identified, indicating viral cocirculation within poultry farms (data not shown).

H5N8 Genotypes

Of the 539 detected HPAI H5 viruses, we characterized 212 viral genomes: 15 from wild birds (Appendix Table 2) and 197 from domestic or captive birds. Phylogenetic analyses of 8 genes indicated that the H5N8 viruses from France formed a monophyletic cluster for only the hemagglutinin, neuraminidase, matrix, and nonstructural genes (a monophyletic cluster has >98% similarity and a bootstrap value of ≥ 75), whereas the PB2, PB1, PA, and NP sequences formed 2 different phylogenetic clusters. We identified 3 genotypes (A–C) in France on

the basis of all segment sequences. Genotype A differed from genotype B in segments PB2, PA, and NP and differed from genotype C in only segment PB1. Genotype A comprised 197 viruses and was a H5N8-A/mute_swan/Croatia/70/2016-like virus (35). Although genotype A was the most common genotype in our study, we found it only in southwestern France (Figure 2). We detected 192 genotype A viruses in poultry but only 5 in wild birds. Genotype B was a A/wild_duck/Poland/82A/2016-like virus (35,36). We found genotype B viruses in northern, western, and eastern France and detected 3 viruses in captive/domestic birds and 5 in wild birds. Genotype C was a A/domestic_goose/Poland/33/2016-like virus (37). We detected 7 genotype C viruses: 2 in captive/domestic birds in southwestern France and 5 in wild birds in eastern France.

Geographic Clustering of Genotype A Viruses

On November 28, 2016, we detected genotype A virus in domestic breeding ducks in southwestern France. In total, we found 496 cases of HPAI

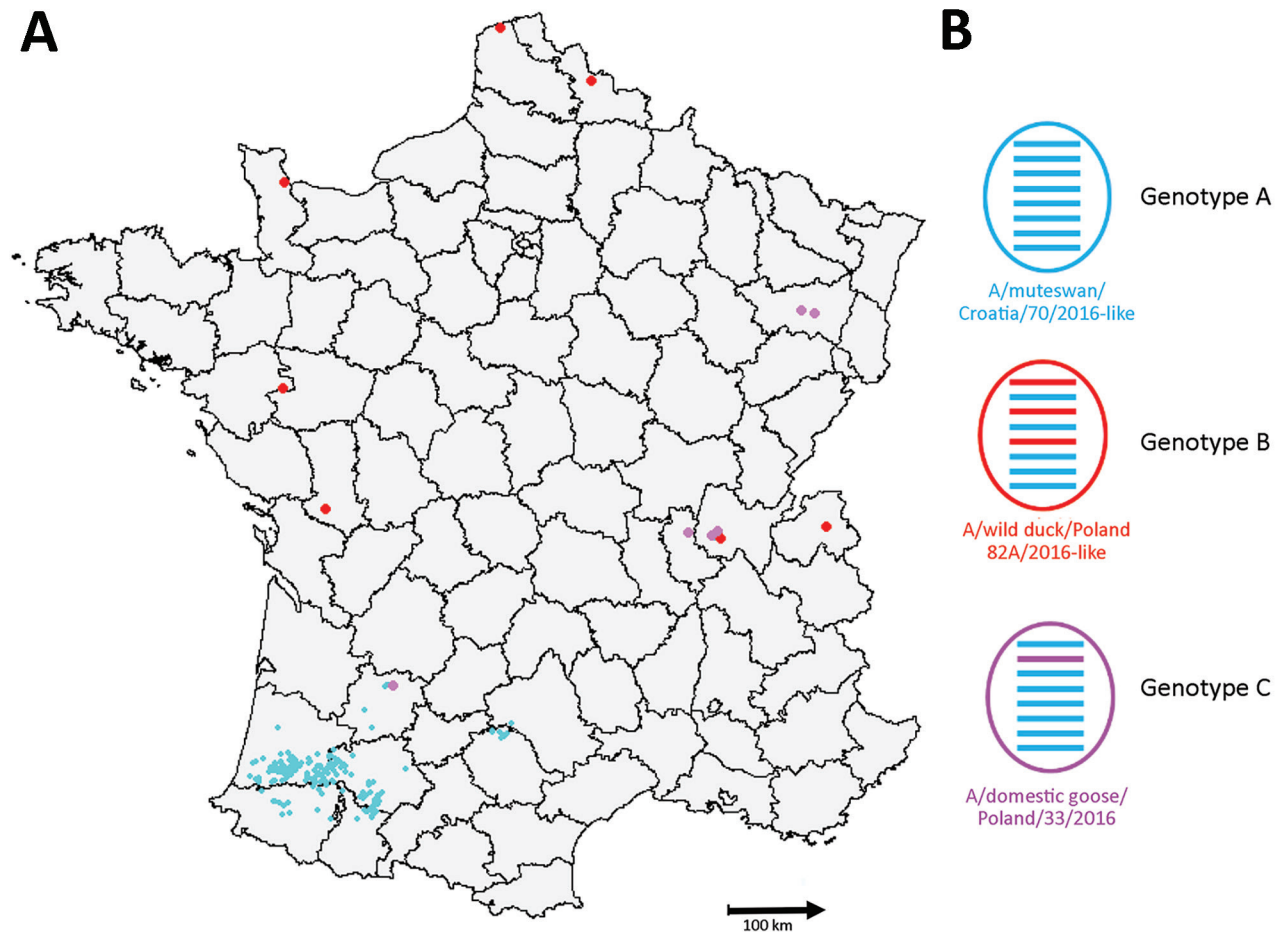


Figure 2. Distribution of the 3 detected genotypes of highly pathogenic avian influenza H5N8 viruses, France, 2016–17. A) Geographic distribution of genotypes. B) Representation of viral genome. Horizontal bars correspond to the 8 gene segments of each characterized genotype. Segments colored according to phylogenetic cluster.

A(H5N8) infection in southwestern France. Of the 496 cases, we determined full genome sequences for 197 (41.25%) viruses, all of which were genotype A. The 197 genomes comprised 5 geographic clusters: geocluster 1 contained 10 viruses in France departments nos. 12 and 81; geocluster 2 contained 5 viruses in department no. 47; geocluster 3 contained 41 viruses mostly in departments nos. 32 and 65; geocluster 4 contained 74 viruses in the east of the department no. 40 and a few viruses in departments nos. 32 and 64; geocluster 5 contained 67 sequences in departments nos. 40 and 64 (Figure 3).

The viruses in geocluster 1 were closely related (Figure 3); the tMRCA was November 16, 2016 (highest posterior density [HPD] 95% CI November 9–23) (Appendix Table 3). The viruses in geocluster 5 had a common ancestor that emerged on January 15, 2017 (HPD 95% CI January 7–23) from geocluster 3 (Appendix Table 4). This date probably corresponds with introduction of HPAI A(H5N8) into geocluster 5; the first case in geocluster 5 was documented in domestic ducks on January 30, 2017 (Figure 4). The first sequences to emerge in geoclusters 2, 3, and 4 were similar; afterwards, the sequences diverged into each geocluster. We did not calculate the viral transmission dates for geoclusters 2, 3, and 4 because these phylogenetic groups were not monophyletic and did not have posterior probabilities >0.8 for their ancestral nodes.

We constructed a phylogenetic tree of the 197 analyzed genomes (Figure 3). The tree had several principle nodes composed of identical sequences; many leaves were linked, indicating the evolution of numerous sequences from the principal nodes. The mean nucleotide difference between 2 related sequences belonging to distinct nodes was ≈ 3.1 mutations (range 1–11 mutations). The mean mutation rate of the complete genome was 6.68×10^{-3} (HPD 95% CI $5.96\text{--}7.43 \times 10^{-3}$) substitutions/site/year.

Dynamic Evolution of Genotype A

We used a Bayesian Skygrid plot to analyze the population growth of H5N8 viruses in southwestern France (Figure 5). The overall population increased during November 2016–January 2017, which corresponds to the period in which moderate viral spread occurred in geoclusters 1 and 2 and more pronounced spread occurred in geoclusters 3 and 4. After this time, we noted an overall population decrease corresponding with the last cases reported in geoclusters 3 and 4. The population dramatically increased during February 2017, when cases began in geocluster 5. The HPAI A(H5N8) population size declined in March 2017.

Discussion

The 2016–17 HPAI A(H5N8) outbreak in Europe affected 1,576 wild birds and 1,134 domestic birds (18).

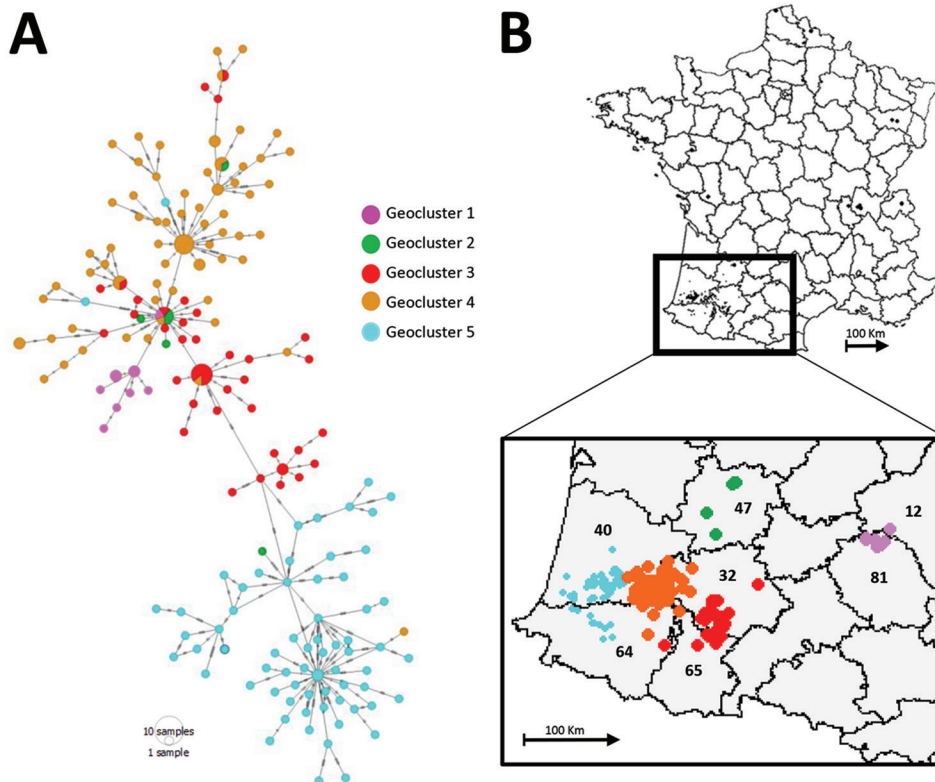


Figure 3. Minimum spanning tree and map of clusters of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. A) Geographic clusters. Number of dashes indicates the number of observed mutations between 2 nodes. Circle size corresponds to the number of identical sequences. B) Geographic repartition of genotype in southwestern France. Inset shows identification numbers of affected departments: 12, Aveyron; 31, Haute-Garonne; 32, Gers; 47, Lot et Garonne; 40, Landes; 64, Pyrénées-Atlantiques; 65, Hautes-Pyrénées. Trees created using PopART (32).

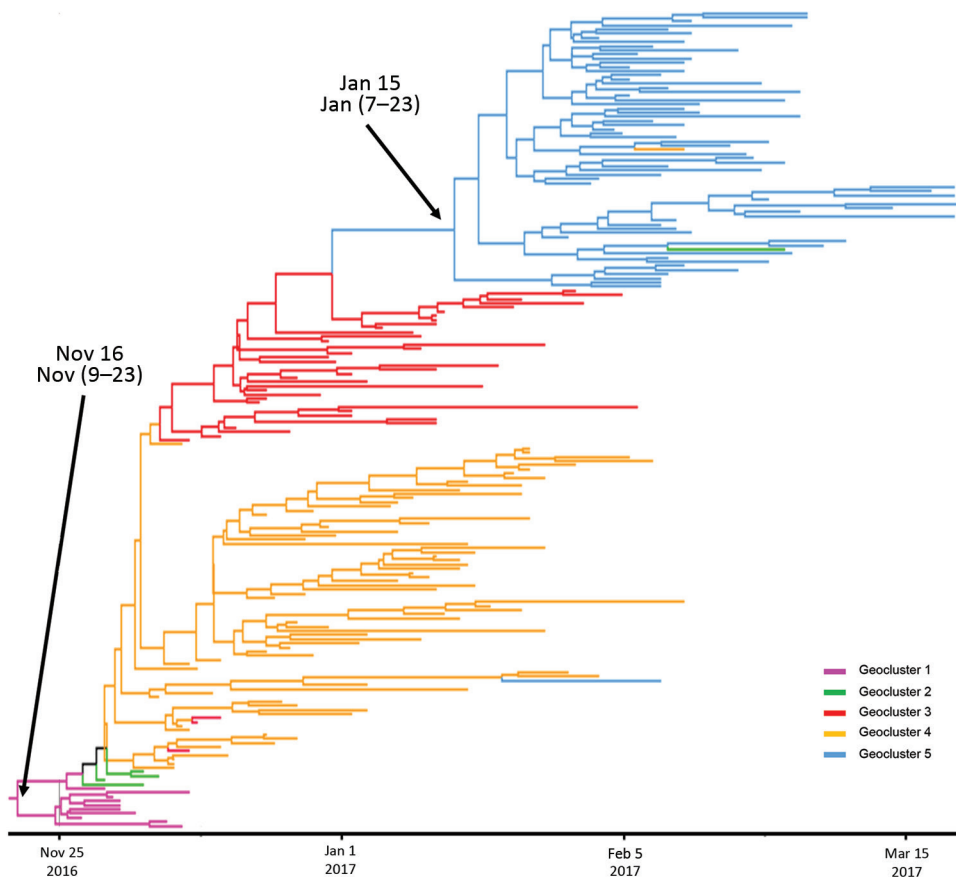


Figure 4. Maximum credibility tree of the 8 concatenated gene segments in highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Tree generated using SDR06 model according to Bayesian method (38). Branch and leaf color indicates geoclusters. The estimated dates of common ancestors and their 95% CIs are indicated for geocluster 1 and geocluster 5.

In France, we identified 3 genotypes that had previously been described elsewhere in Europe (19,35–7), indicating that H5N8 was introduced into France ≥ 3 times during November 2016–April 2017. We found sporadic cases of genotypes B and C, mostly in wild birds. We found 197 viruses of genotype A, almost all of which were in domestic ducks in southwestern France. Only 2 viruses of genotype A were in backyard poultry, an observation that corresponds to the findings of Souvestre et al. (39), which showed the minor role of backyards in the H5N8 transmission dynamic. Of the 6 genotypes characterized during this outbreak in Europe, 3 genotypes resemble the sequences now described in France (i.e., genotype A corresponds with reassortants 6-like, B with reassortants 3-like, and C with reassortants 7-like) (19).

Similar sequences to genotype A viruses were identified in Croatia, Italy, Belgium, Poland, and the Czech Republic; they also were found in domestic ducks in Hungary (19). France and Hungary are the main producers of foie gras in Europe. Areas with high duck farm density (34) had an increased number of H5N8 cases in domestic birds during this outbreak (18,19). The H5N8 sequences found in Hungary are

closely related to the genotype A viruses described in this study, an observation that might indicate an epidemiologic link between these 2 regions. Alternatively, the viral similarity could have been caused by the common use of mule ducks for foie gras, which might be more susceptible to genotype A than other H5N8 viruses.

All genotype A viruses found in France were closely related and formed a monophyletic cluster, strongly suggesting that this genotype was introduced only once into southwestern France. Genotype A viruses might have spread among domestic duck farms in a multistep process. First, genotype A viruses were introduced into southwestern France, where they spread and formed geocluster 1. According to the tMRCA values, this introduction probably occurred around November 16, 2016. Second, the apparent transfer of infected ducks enabled H5N8 to spread to other areas of southwest France, prompting the formation of geoclusters 2, 3, and 4 (40). Third, the virus spread among farms in newly affected areas, possibly through airborne transmission or movements of animals, materials, or personnel among farms, as suggested by Andronico et al. (41). Fourth, the virus

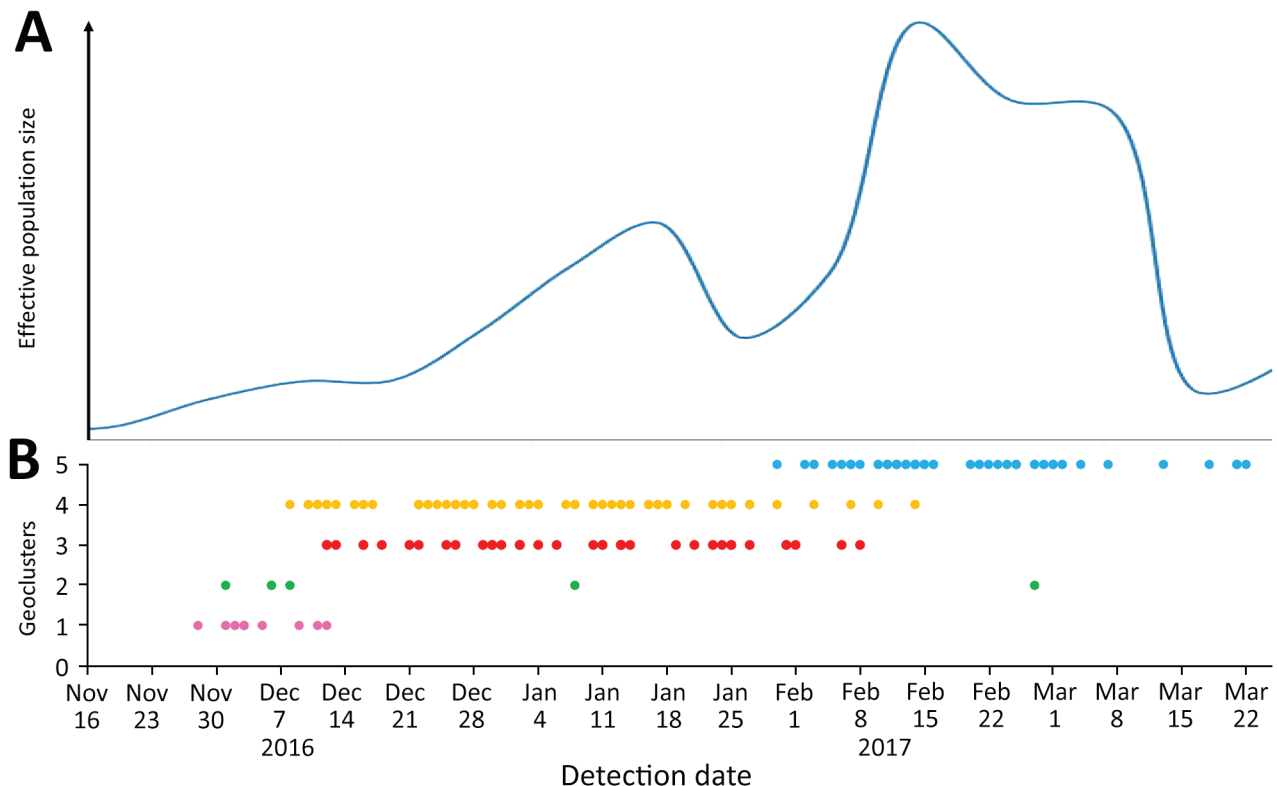


Figure 5. Evolution of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. A) Bayesian Skygrid plot of viral population size over time. B) Timeline of cases of H5N8 genotype A. Pink indicates geocluster 1; green indicates geocluster 2; red indicates geocluster 3; orange indicates geocluster 4; blue indicates geocluster 5.

entered the geographic area corresponding to geocluster 5. This geocluster included viral genome sequences closely related to those of geocluster 3. This finding was unexpected because the geographic area of geocluster 5 is closer to that of geocluster 4 than geocluster 3. The low variability among geocluster 5 sequences suggests that the virus was introduced through a single viral transmission. We estimated that this event occurred around January 15, 2017, approximately 2 weeks before we first sequenced virus in this geocluster (i.e., January 30, 2017). This delay suggests that we might not have sampled all cases. In addition, the precision of our model could have been increased by using path and stepping-stone sampling methods. The single introduction seems to have been the origin of all subsequent infections in this area. This long-range viral transmission could have occurred through animal transport or the movement of wild birds. Once this new area was infected, the virus spread among nearby farms, resulting in the formation of geocluster 5.

Our results correspond with the estimation of the effective population size of the HPAI A(H5N8) viruses in southwestern France. The first increase of the viral population coincided with the emergence of

geoclusters 3 and 4. The subsequent population decrease might reflect governmental actions to control viral dissemination, such as the preventive culling of poultry and ducks in farms with confirmed infection. In addition, the 5 geoclusters identified in this study correspond with the geoclusters characterized by Guinat et al. (20) on the basis of the dates and locations of clinical reports. According to Guinat et al., the depopulation of poultry farms and restrictions on movement of animals, materials, or personnel among farms could have substantially reduced viral spread within each geocluster. The second increase in the viral population coincided with the introduction of H5N8 into a new area (i.e., that of geocluster 5) with a high density of poultry farms (41). These results highlight the importance of controlling poultry movements to prevent viral spread, especially because these movements were identified as a risk factor for transmission in southwest France during this outbreak (42). Our data suggest that viral spread was directly related to the density of duck holdings. For example, the virus was effectively restrained in geoclusters 1 and 2, which corresponded to areas of low duck-holding density. The other 3 geoclusters had a

higher density of duck farms, facilitating the local (inside the same geocluster) and long distance (between geoclusters) spreads of the virus. These results should be further combined with the epidemiologic data and Bayesian discrete trait phylogeography analysis to identify transmission factors.

In conclusion, during winter 2016–17, Europe faced a large outbreak of HPAI A(H5N8). Three viral genotypes were detected in France, but only genotype A caused dramatic economic losses. In southwestern France, a major producer of foie gras, genotype A viruses were detected in 5 separate geographic clusters. Our data show that local dissemination and long-distance transmission contributed to the severity of the outbreak, especially in areas of high duck-holding density. This study highlights the importance of limiting introduction of infected birds into a disease-free area. Implementing control measures for infected flocks is crucial to avoiding the spread of AIVs.

Acknowledgments

We thank the veterinarians, farmers, and Office National de la Chasse de la et de la Faune Sauvage staff involved for the collection of field samples. We are also grateful to the screening laboratories for the initial screening samples and to the local Directions Départementales de la Protection des Populations and central Direction Générale de l'Alimentation official veterinary services for the outbreak management.

This study was supported by Fonds de la Recherche pour l'Influenza Aviaire 2019 from the French Ministry of Agriculture.

About the Author

Mr. Briand is a scientist at the French Reference Laboratory for Avian Influenza and Newcastle Disease. His research interests include phylogeny, virology, and molecular epidemiology, especially for avian influenza virus and Newcastle disease virus.

References

- Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus AD, Fouchier RA. Global patterns of influenza A virus in wild birds. *Science*. 2006;312:384–8. <https://doi.org/10.1126/science.1122438>
- Nao N, Yamagishi J, Miyamoto H, Igarashi M, Manzoor R, Ohnuma A, et al. Genetic predisposition to acquire a polybasic cleavage site for highly pathogenic avian influenza virus hemagglutinin. *MBio*. 2017;8:e02298–16. <https://doi.org/10.1128/mBio.02298-16>
- OFFLU. Influenza A cleavage sites. 2020 Jul 8 [cited 2020 Nov 30]. http://www.offlu.net/fileadmin/home/en/resource-centre/pdf/Influenza_A_Cleavage_Sites.pdf
- Pantin-Jackwood MJ, Swayne DE. Pathogenesis and pathobiology of avian influenza virus infection in birds. *Rev Sci Tech*. 2009;28:113–36. <https://doi.org/10.20506/rst.28.1.1869>
- Lee DH, Criado MF, Swayne DE. Pathobiological origins and evolutionary history of highly pathogenic avian influenza viruses. *Cold Spring Harb Perspect Med*. 2020 Jan 21 [Epub ahead of print]. <https://doi.org/10.1101/cshperspect.a038679>
- European Commission. Council directive 2005/94/EC on community measures for the control of avian influenza and repealing directive 92/40/EEC. 2019 Jan 1 [cited 2020 Nov 30]. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32005L0094>
- World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization H5N1 Evolution Working Group. Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). *Emerg Infect Dis*. 2008;14:e1. <https://doi.org/10.3201/eid1407.071681>
- Fusaro A, Zecchin B, Vrancken B, Abolnik C, Ademun R, Alassane A, et al. Disentangling the role of Africa in the global spread of H5 highly pathogenic avian influenza. *Nat Commun*. 2019;10:5310. <https://doi.org/10.1038/s41467-019-13287-y>
- Saito T, Tanikawa T, Uchida Y, Takemae N, Kanehira K, Tsunekuni R. Intracontinental and intercontinental dissemination of Asian H5 highly pathogenic avian influenza virus (clade 2.3.4.4) in the winter of 2014–2015. *Rev Med Virol*. 2015;25:388–405. <https://doi.org/10.1002/rmv.1857>
- Lee DH, Torchetti MK, Winker K, Ip HS, Song CS, Swayne DE. Intercontinental spread of Asian-origin H5N8 to North America through Beringia by migratory birds. *J Virol*. 2015;89:6521–4. <https://doi.org/10.1128/JVI.00728-15>
- Lee DH, Bertran K, Kwon JH, Swayne DE. Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. *J Vet Sci*. 2017;18:269–80. <https://doi.org/10.4142/jvs.2017.18.S1.269>
- Cattoli G, Fusaro A, Monne I, Capua I. H5N1 virus evolution in Europe – an updated overview. *Viruses*. 2009;1:1351–63. <https://doi.org/10.3390/v1031351>
- Global Consortium for H5N8 and Related Influenza Viruses. Role for migratory wild birds in the global spread of avian influenza H5N8. *Science*. 2016;354:213–7. <https://doi.org/10.1126/science.aaf8852>
- King J, Schulze C, Engelhardt A, Hlinak A, Lennermann SL, Rigbers K, et al. Novel HPAIV H5N8 reassortant (clade 2.3.4.4b) detected in Germany. *Viruses*. 2020;12:281. <https://doi.org/10.3390/v12030281>
- Hill NJ, Hussein IT, Davis KR, Ma EJ, Spivey TJ, Ramey AM, et al. Reassortment of influenza A viruses in wild birds in Alaska before H5 clade 2.3.4.4 outbreaks. *Emerg Infect Dis*. 2017;23:654–7. <https://doi.org/10.3201/eid2304.161668>
- Antigua KJC, Choi WS, Baek YH, Song MS. The emergence and decennary distribution of clade 2.3.4.4 HPAI H5Nx. *Microorganisms*. 2019;7:156. <https://doi.org/10.3390/microorganisms7060156>
- Pohlmann A, Starick E, Grund C, Höper D, Strebellow G, Globig A, et al. Swarm incursions of reassortants of highly pathogenic avian influenza virus strains H5N8 and H5N5, clade 2.3.4.4b, Germany, winter 2016/17. *Sci Rep*. 2018;8:15. <https://doi.org/10.1038/s41598-017-16936-8>
- Brown I, Kuiken T, Mulatti P, Smietanka K, Staubach C, Stroud D, et al.; European Food Safety Authority; European Centre for Disease Prevention and Control; European Union Reference Laboratory for Avian influenza.

- Avian influenza overview September–November 2017. *EFSA J.* 2017;15:e05141.
19. Lycett SJ, Pohlmann A, Staubach C, Caliendo V, Woolhouse M, Beer M, et al. Global Consortium for H5N8 and Related Influenza Viruses. Genesis and spread of multiple reassortants during the 2016/2017 H5 avian influenza epidemic in Eurasia. *Proc Natl Acad Sci U S A.* 2020; 117:20814–25. <https://doi.org/10.1073/pnas.2001813117>
 20. Guinat C, Nicolas G, Vergne I, Bronner A, Durand B, Courcoul A, et al. Spatio-temporal patterns of highly pathogenic avian influenza virus subtype H5N8 spread, France, 2016 to 2017. *Euro Surveill.* 2018;23. <https://doi.org/10.2807/1560-7917.ES.2018.23.26.1700791>
 21. Bronner A, Niqueux E, Schmitz A, Le Bouquin S, Huneau-Salaün A, Guinat C, et al. Description of the highly pathogenic avian influenza episode in France in 2016–2017 [in French]. *Bulletin épidémiologique, santé animale et alimentation.* 2017;79:13–7.
 22. Slomka MJ, Coward VJ, Banks J, Löndt BZ, Brown IH, Voermans J, et al. Identification of sensitive and specific avian influenza polymerase chain reaction methods through blind ring trials organized in the European Union. *Avian Dis.* 2007;51:227–34. <https://doi.org/10.1637/7674-063006R1.1>
 23. Zhou B, Donnelly ME, Scholes DT, St George K, Hatta M, Kawaoka Y, et al. Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and swine origin human influenza A viruses. *J Virol.* 2009;83:10309–13. <https://doi.org/10.1128/JVI.01109-09>
 24. Briand FX, Schmitz A, Ogor K, Le Prioux A, Guillou-Cloarec C, Guillemoto C, et al. Emerging highly pathogenic H5 avian influenza viruses in France during winter 2015/16: phylogenetic analyses and markers for zoonotic potential. *Euro Surveill.* 2017;22:30473. <https://doi.org/10.2807/1560-7917.ES.2017.22.9.30473>
 25. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* 2012;9:357–9. <https://doi.org/10.1038/nmeth.1923>
 26. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 2016;33:1870–4. <https://doi.org/10.1093/molbev/msw054>
 27. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol.* 2015;1:vev003. <https://doi.org/10.1093/ve/vev003>
 28. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol.* 2012;29:1969–73. <https://doi.org/10.1093/molbev/mss075>
 29. Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA, Alekseyenko AV. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Mol Biol Evol.* 2012;29:2157–67. <https://doi.org/10.1093/molbev/mss084>
 30. Hill V, Baele G. Bayesian estimation of past population dynamics in BEAST 1.10 using the Skygrid coalescent model. *Mol Biol Evol.* 2019;36:msz172. <https://doi.org/10.1093/molbev/msz172>
 31. Lanfear R, Hua X, Warren DL. Estimating the effective sample size of tree topologies from Bayesian phylogenetic analyses. *Genome Biol Evol.* 2016;8:2319–32. <https://doi.org/10.1093/gbe/evw171>
 32. Vaughan TG. IcyTree: rapid browser-based visualization for phylogenetic trees and networks. *Bioinformatics.* 2017;33:2392–4. <https://doi.org/10.1093/bioinformatics/btx155>
 33. Leigh J, Bryant D. POPART: full-feature software for haplotype network construction. *Methods Ecol Evol.* 2015;6:1110–6. <https://doi.org/10.1111/2041-210X.12410>
 34. Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. Assessing avian influenza risk levels and their evolution [in French]. 2017 [cited 2017 Nov 07]. <https://www.anses.fr/fr/system/files/SABA2017SA0203-partie2.pdf>
 35. Fusaro A, Monne I, Mulatti P, Zecchin B, Bonfanti L, Ormelli S, et al. Genetic diversity of highly pathogenic avian influenza A(H5N8/H5N5) viruses in Italy, 2016–17. *Emerg Infect Dis.* 2017;23:1543–7. <https://doi.org/10.3201/eid2309.170539>
 36. Świątoń E, Śmietanka K. Phylogenetic and molecular analysis of highly pathogenic avian influenza H5N8 and H5N5 viruses detected in Poland in 2016–2017. *Transbound Emerg Dis.* 2018;65:1664–70. <https://doi.org/10.1111/tbed.12924>
 37. Savić V. Novel reassortant clade 2.3.4.4 avian influenza A(H5N5) virus in wild birds and poultry, Croatia, 2016–2017. *Vet Arh.* 2017;87:377–96. <https://doi.org/10.24099/vet.arhiv.170509>
 38. Shapiro B, Rambaut A, Drummond AJ. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol Biol Evol.* 2006;23:7–9. <https://doi.org/10.1093/molbev/msj021>
 39. Souvestre M, Guinat C, Niqueux E, Robertet L, Croville G, Paul M, et al. Role of backyard flocks in transmission dynamics of highly pathogenic avian influenza A(H5N8) clade 2.3.4.4, France, 2016–2017. *Emerg Infect Dis.* 2019;25:551–4. <https://doi.org/10.3201/eid2503.181040>
 40. Moisson M, Hamon M, Malhere C, Donguy M, Niqueux E, Scoizec A, et al. The situation of HP H5N8 avian influenza in France as of 05/12/2016. 2016 [cited 2016 Dec 06]. <https://www.plateforme-esa.fr/article/situation-de-l-influenza-aviaire-hp-h5n8-en-france-au-05122016>
 41. Andronico A, Courcoul A, Bronner A, Scoizec A, Lebouquin-Leneveu S, Guinat C, et al. Highly pathogenic avian influenza H5N8 in south-west France 2016–2017: a modeling study of control strategies. *Epidemics.* 2019; 28:100340. <https://doi.org/10.1016/j.epidem.2019.03.006>
 42. Guinat C, Artois J, Bronner A, Guérin JL, Gilbert M, Paul MC. Duck production systems and highly pathogenic avian influenza H5N8 in France, 2016–2017. *Sci Rep.* 2019;9:6177. <https://doi.org/10.1038/s41598-019-42607-x>

Address for correspondence: François-Xavier Briand, Anses, Ploufragan-Plouzané-Niort Laboratory, Avian and Rabbit Virology Immunology and Parasitology Unit, National Reference Laboratory for Avian Influenza, BP53, 22440 Ploufragan, France; email: francois-xavier.briand@anses.fr

Highly Pathogenic Avian Influenza A(H5N8) Virus Spread by Short- and Long-Range Transmission, France, 2016–17

Appendix Table 1. Accession nos. of highly pathogenic influenza A(H5N8) viruses, France, 2016–2017*

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
161104	<i>Mareca strepera</i>	2016 Nov 26	62	Marck	EPI86 9684	EPI86 9685	EPI86 9686	EPI86 9687	EPI86 9688	EPI86 9689	EPI86 9690	EPI86 9691
161108	domestic duck†	2016 Nov 29	81	Almayrac	EPI86 9806	EPI86 9807	EPI86 9808	EPI86 9809	EPI86 9810	EPI86 9811	EPI86 9812	EPI86 9813
161113	domestic duck	2016 Dec 1	81	Almayrac	MN87 4281	MN87 4491	MN87 4701	MN87 4911	MN87 5121	MN87 5331	MN87 5541	MN87 5751
161116	domestic duck	2016 Dec 1	47	Monbahus	MN87 4282	MN87 4492	MN87 4702	MN87 4912	MN87 5122	MN87 5332	MN87 5542	MN87 5752
161137	domestic duck	2016 Dec 2	81	Lacappelle-Ségalar	MN87 4283	MN87 4493	MN87 4703	MN87 4913	MN87 5123	MN87 5333	MN87 5543	MN87 5753
161141	domestic duck	2016 Dec 3	81	Mouzieys-Panens	MN87 4284	MN87 4494	MN87 4704	MN87 4914	MN87 5124	MN87 5334	MN87 5544	MN87 5754
161142	domestic duck	2016 Dec 3	81	Monestiés	MN87 4285	MN87 4495	MN87 4705	MN87 4915	MN87 5125	MN87 5335	MN87 5545	MN87 5755
161143	domestic duck	2016 Dec 3	81	Monestiés	MN87 4286	MN87 4496	MN87 4706	MN87 4916	MN87 5126	MN87 5336	MN87 5546	MN87 5756
161147	domestic duck	2016 Dec 5	81	Monestiés	MN87 4287	MN87 4497	MN87 4707	MN87 4917	MN87 5127	MN87 5337	MN87 5547	MN87 5757
161173	domestic duck	2016 Dec 6	47	Monbahus	MN87 4288	MN87 4498	MN87 4708	MN87 4918	MN87 5128	MN87 5338	MN87 5548	MN87 5758
161174	domestic duck	2016 Dec 6	47	Monbahus	MN87 4289	MN87 4499	MN87 4709	MN87 4919	MN87 5129	MN87 5339	MN87 5549	MN87 5759
161176	<i>Larus argentatus</i>	2017 Nov 23	74	Cluses	MN87 4481	MN87 4691	MN87 4901	MN87 5111	MN87 5321	MN87 5531	MN87 5741	MN87 5951
161182	domestic duck	2016 Dec 8	47	Monbahus	MN87 4290	MN87 4500	MN87 4710	MN87 4920	MN87 5130	MN87 5340	MN87 5550	MN87 5760
161207	domestic duck	2016 Dec 9	12	Tayrac	MN87 4291	MN87 4501	MN87 4711	MN87 4921	MN87 5131	MN87 5341	MN87 5551	MN87 5761
161212	domestic duck	2016 Dec 8	32	Segos	MN87 4292	MN87 4502	MN87 4712	MN87 4922	MN87 5132	MN87 5342	MN87 5552	MN87 5762
161224	domestic duck	2016 Dec 11	32	Avéron-Bergelle	MN87 4293	MN87 4503	MN87 4713	MN87 4923	MN87 5133	MN87 5343	MN87 5553	MN87 5763
161225	domestic duck	2016 Dec 11	32	Eauze	MN87 4294	MN87 4504	MN87 4714	MN87 4924	MN87 5134	MN87 5344	MN87 5554	MN87 5764
161227	domestic duck	2016 Dec 10	32	Caupenne-d'Armagnac	MN87 4295	MN87 4505	MN87 4715	MN87 4925	MN87 5135	MN87 5345	MN87 5555	MN87 5765
161228	domestic duck	2016 Dec 10	32	Caupenne-d'Armagnac	MN87 4296	MN87 4506	MN87 4716	MN87 4926	MN87 5136	MN87 5346	MN87 5556	MN87 5766
161229	domestic duck	2016 Dec 10	32	Caupenne-d'Armagnac	MN87 4297	MN87 4507	MN87 4717	MN87 4927	MN87 5137	MN87 5347	MN87 5557	MN87 5767
161230	domestic duck	2016 Dec 12	40	Eugénie-les-Bains	MN87 4298	MN87 4508	MN87 4718	MN87 4928	MN87 5138	MN87 5348	MN87 5558	MN87 5768
161231	domestic duck	2016 Dec 12	64	Espoeey	MN87 4299	MN87 4509	MN87 4719	MN87 4929	MN87 5139	MN87 5349	MN87 5559	MN87 5769
161233	domestic duck	2016 Dec 12	32	Mansempuy	MN87 4300	MN87 4510	MN87 4720	MN87 4930	MN87 5140	MN87 5350	MN87 5560	MN87 5770
161234	domestic duck	2016 Dec 12	32	Mauléon-d'Armagnac	MN87 4301	MN87 4511	MN87 4721	MN87 4931	MN87 5141	MN87 5351	MN87 5561	MN87 5771
161239	domestic duck	2016 Dec 11	12	Tayrac	MN87 4302	MN87 4512	MN87 4722	MN87 4932	MN87 5142	MN87 5352	MN87 5562	MN87 5772
161240	domestic duck	2016 Dec 12	81	Saint-Martin-Laguépie	MN87 4303	MN87 4513	MN87 4723	MN87 4933	MN87 5143	MN87 5353	MN87 5563	MN87 5773

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
161242	domestic duck	2016 Dec 13	32	Saint-Medard	MN87 4304	MN87 4514	MN87 4724	MN87 4934	MN87 5144	MN87 5354	MN87 5564	MN87 5774
161243	domestic duck	2016 Dec 13	40	Lussagnet-Lusson	MN87 4305	MN87 4515	MN87 4725	MN87 4935	MN87 5145	MN87 5355	MN87 5565	MN87 5775
161256	domestic duck	2016 Dec 16	32	Monlezun	MN87 4306	MN87 4516	MN87 4726	MN87 4936	MN87 5146	MN87 5356	MN87 5566	MN87 5776
161263	domestic duck	2016 Dec 15	32	D'Armagnac Bernède	MN87 4307	MN87 4517	MN87 4727	MN87 4937	MN87 5147	MN87 5357	MN87 5567	MN87 5777
161271	domestic duck	2016 Dec 16	32	Saint-Medard	MN87 4308	MN87 4518	MN87 4728	MN87 4938	MN87 5148	MN87 5358	MN87 5568	MN87 5778
161275	domestic duck	2016 Dec 18	32	Bars	MN87 4309	MN87 4519	MN87 4729	MN87 4939	MN87 5149	MN87 5359	MN87 5569	MN87 5779
161277	domestic duck	2016 Dec 17	32	Laujuzan	MN87 4310	MN87 4520	MN87 4730	MN87 4940	MN87 5150	MN87 5360	MN87 5570	MN87 5780
161298	domestic duck	2016 Dec 16	32	nonprécisé	MN87 4311	MN87 4521	MN87 4731	MN87 4941	MN87 5151	MN87 5361	MN87 5571	MN87 5781
161323	<i>Mareca penelope</i>	2016 Dec 18	50	Sainte-Marie-du-Mont	MN87 4479	MN87 4689	MN87 4899	MN87 5109	MN87 5319	MN87 5529	MN87 5739	MN87 5949
161383	domestic duck	2016 Dec 21	65	Fontrailles	MN87 4312	MN87 4522	MN87 4732	MN87 4942	MN87 5152	MN87 5362	MN87 5572	MN87 5782
161443	domestic duck	2016 Dec 22	32	Barcugnan	MN87 4313	MN87 4523	MN87 4733	MN87 4943	MN87 5153	MN87 5363	MN87 5573	MN87 5783
161444	domestic duck	2016 Dec 23	40	Perquie	MN87 4314	MN87 4524	MN87 4734	MN87 4944	MN87 5154	MN87 5364	MN87 5574	MN87 5784
161445	domestic duck	2016 Dec 22	32	Monguilhen	MN87 4315	MN87 4525	MN87 4735	MN87 4945	MN87 5155	MN87 5365	MN87 5575	MN87 5785
161449	domestic duck	2016 Dec 22	32	Barcelonne-du-Gers	MN87 4316	MN87 4526	MN87 4736	MN87 4946	MN87 5156	MN87 5366	MN87 5576	MN87 5786
161450	domestic duck	2016 Dec 22	40	Saint-Agnet	MN87 4317	MN87 4527	MN87 4737	MN87 4947	MN87 5157	MN87 5367	MN87 5577	MN87 5787
161455	domestic duck	2016 Dec 23	40	Duhort-Bachen	MN87 4318	MN87 4528	MN87 4738	MN87 4948	MN87 5158	MN87 5368	MN87 5578	MN87 5788
161456	domestic duck	2016 Dec 26	32	Monguilhem	MN87 4319	MN87 4529	MN87 4739	MN87 4949	MN87 5159	MN87 5369	MN87 5579	MN87 5789
161457	domestic duck	2016 Dec 22	32	Duffort	MN87 4320	MN87 4530	MN87 4740	MN87 4950	MN87 5160	MN87 5370	MN87 5580	MN87 5790
161473	domestic duck	2016 Dec 24	32	Eauze	MN87 4321	MN87 4531	MN87 4741	MN87 4951	MN87 5161	MN87 5371	MN87 5581	MN87 5791
161475	domestic duck	2016 Dec 26	40	Duhort-Bachen	MN87 4322	MN87 4532	MN87 4742	MN87 4952	MN87 5162	MN87 5372	MN87 5582	MN87 5792
161476	domestic duck	2016 Dec 16	40	Duhort-Bachen	MN87 4323	MN87 4533	MN87 4743	MN87 4953	MN87 5163	MN87 5373	MN87 5583	MN87 5793
161477	domestic duck	2016 Dec 25	40	Aire-sur-l'Adour	MN87 4324	MN87 4534	MN87 4744	MN87 4954	MN87 5164	MN87 5374	MN87 5584	MN87 5794
161478	domestic duck	2016 Dec 25	32	Tillac	MN87 4325	MN87 4535	MN87 4745	MN87 4955	MN87 5165	MN87 5375	MN87 5585	MN87 5795
161497	domestic duck	2016 Dec 27	32	Segos	MN87 4326	MN87 4536	MN87 4746	MN87 4956	MN87 5166	MN87 5376	MN87 5586	MN87 5796
161498	domestic duck	2016 Dec 28	40	Duhort-Bachen	MN87 4327	MN87 4537	MN87 4747	MN87 4957	MN87 5167	MN87 5377	MN87 5587	MN87 5797
161500	domestic duck	2016 Dec 26	65	Guizerix	MN87 4328	MN87 4538	MN87 4748	MN87 4958	MN87 5168	MN87 5378	MN87 5588	MN87 5798
161501	domestic duck	2016 Dec 26	32	Eauze	MN87 4329	MN87 4539	MN87 4749	MN87 4959	MN87 5169	MN87 5379	MN87 5589	MN87 5799
161577	domestic duck	2016 Dec 27	40	Aire-sur-l'Adour	MN87 4330	MN87 4540	MN87 4750	MN87 4960	MN87 5170	MN87 5380	MN87 5590	MN87 5800
161585	<i>Gallus Gallus</i>	2016 Dec 28	79	Fors	MN87 4478	MN87 4688	MN87 4898	MN87 5108	MN87 5318	MN87 5528	MN87 5738	MN87 5948
161587	domestic duck	2016 Dec 28	40	Saint-Agnet	MN87 4331	MN87 4541	MN87 4751	MN87 4961	MN87 5171	MN87 5381	MN87 5591	MN87 5801
161600	domestic duck	2016 Dec 29	32	Sauviac	MN87 4332	MN87 4542	MN87 4752	MN87 4962	MN87 5172	MN87 5382	MN87 5592	MN87 5802
161601	domestic duck	2016 Dec 30	40	Renung	MN87 4333	MN87 4543	MN87 4753	MN87 4963	MN87 5173	MN87 5383	MN87 5593	MN87 5803
170013	domestic duck	2016 Dec 30	32	Viella	MN87 4334	MN87 4544	MN87 4754	MN87 4964	MN87 5174	MN87 5384	MN87 5594	MN87 5804
170016	domestic duck	2016 Dec 30	32	Saint-Martin	MN87 4335	MN87 4545	MN87 4755	MN87 4965	MN87 5175	MN87 5385	MN87 5595	MN87 5805

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
170017	domestic duck	2016 Dec 30	32	Saint-Michel	MN87 4336	MN87 4546	MN87 4756	MN87 4966	MN87 5176	MN87 5386	MN87 5596	MN87 5806
170018	domestic duck	2017 Jan 2	32	L'Isle-de-Noé	MN87 4337	MN87 4547	MN87 4757	MN87 4967	MN87 5177	MN87 5387	MN87 5597	MN87 5807
170030	domestic duck	2016 Dec 31	32	Belloc-Saint-Clamens	MN87 4338	MN87 4548	MN87 4758	MN87 4968	MN87 5178	MN87 5388	MN87 5598	MN87 5808
170031	domestic duck	2017 Jan 2	32	Sainte-Christie-d'Armagnac	MN87 4339	MN87 4549	MN87 4759	MN87 4969	MN87 5179	MN87 5389	MN87 5599	MN87 5809
170032	domestic duck	2017 Jan 4	32	Sauviac	MN87 4340	MN87 4550	MN87 4760	MN87 4970	MN87 5180	MN87 5390	MN87 5600	MN87 5810
170033	domestic duck	2016 Dec 31	32	Magnan	MN87 4341	MN87 4551	MN87 4761	MN87 4971	MN87 5181	MN87 5391	MN87 5601	MN87 5811
170034	domestic duck	2017 Jan 3	32	Le Houga	MN87 4342	MN87 4552	MN87 4762	MN87 4972	MN87 5182	MN87 5392	MN87 5602	MN87 5812
170036	domestic duck	2017 Jan 02	32	Montesquiou	MN87 4343	MN87 4553	MN87 4763	MN87 4973	MN87 5183	MN87 5393	MN87 5603	MN87 5813
170038	domestic duck	2017 Jan 2	32	Sauviac	MN87 4344	MN87 4554	MN87 4764	MN87 4974	MN87 5184	MN87 5394	MN87 5604	MN87 5814
170063	<i>Gallus Gallus</i>	2017 Jan 4	40	Saint-Agnet	MN87 4345	MN87 4555	MN87 4765	MN87 4975	MN87 5185	MN87 5395	MN87 5605	MN87 5815
170064	domestic duck	2017 Jan 4	32	Nogaro	MN87 4346	MN87 4556	MN87 4766	MN87 4976	MN87 5186	MN87 5396	MN87 5606	MN87 5816
170067	domestic duck	2017 Jan 4	32	Le Houga	MN87 4347	MN87 4557	MN87 4767	MN87 4977	MN87 5187	MN87 5397	MN87 5607	MN87 5817
170098	domestic duck	2017 Jan 2	32	Saint-Medard	MN87 4348	MN87 4558	MN87 4768	MN87 4978	MN87 5188	MN87 5398	MN87 5608	MN87 5818
170117	domestic duck	2017 Jan 8	40	Miramont-Sensacq	MN87 4349	MN87 4559	MN87 4769	MN87 4979	MN87 5189	MN87 5399	MN87 5609	MN87 5819
170118	domestic duck	2017 Jan 6	65	Coussan	MN87 4350	MN87 4560	MN87 4770	MN87 4980	MN87 5190	MN87 5400	MN87 5610	MN87 5820
170166	swan	2017 Jan 10	1	Bouligneux	MN87 4486	MN87 4696	MN87 4906	MN87 5116	MN87 5326	MN87 5536	MN87 5746	MN87 5956
170176	<i>Gallus Gallus</i>	2017 Jan 7	40	Saint-Agnet	MN87 4351	MN87 4561	MN87 4771	MN87 4981	MN87 5191	MN87 5401	MN87 5611	MN87 5821
170177	domestic duck	2017 Jan 8	40	Mant	MN87 4352	MN87 4562	MN87 4772	MN87 4982	MN87 5192	MN87 5402	MN87 5612	MN87 5822
170178	domestic duck	2017 Jan 8	40	Arboucave	MN87 4353	MN87 4563	MN87 4773	MN87 4983	MN87 5193	MN87 5403	MN87 5613	MN87 5823
170180	duck	2017 Jan 12	47	Nérac	MN87 4354	MN87 4564	MN87 4774	MN87 4984	MN87 5194	MN87 5404	MN87 5614	MN87 5824
170245	domestic duck	2017 Jan 10	40	Sarraziat	MN87 4355	MN87 4565	MN87 4775	MN87 4985	MN87 5195	MN87 5405	MN87 5615	MN87 5825
170249	domestic duck	2017 Jan 10	32	Aux-Aussat	MN87 4356	MN87 4566	MN87 4776	MN87 4986	MN87 5196	MN87 5406	MN87 5616	MN87 5826
170250	domestic duck	2017 Jan 10	40	Miramont-Sensacq	MN87 4357	MN87 4567	MN87 4777	MN87 4987	MN87 5197	MN87 5407	MN87 5617	MN87 5827
170278	<i>Meleagris gallopavo</i>	2017 Jan 11	32	Monclar-sur-Losse	MN87 4358	MN87 4568	MN87 4778	MN87 4988	MN87 5198	MN87 5408	MN87 5618	MN87 5828
170280	domestic duck	2017 Jan 11	32	Saint-Michel	MN87 4359	MN87 4569	MN87 4779	MN87 4989	MN87 5199	MN87 5409	MN87 5619	MN87 5829
170284	domestic duck	2017 Jan 11	40	site de Larrivière	MN87 4360	MN87 4570	MN87 4780	MN87 4990	MN87 5200	MN87 5410	MN87 5620	MN87 5830
170308	domestic duck	2017 Jan 13	32	Laas	MN87 4361	MN87 4571	MN87 4781	MN87 4991	MN87 5201	MN87 5411	MN87 5621	MN87 5831
170309	domestic duck	2017 Jan 13	32	Saint-Arailles	MN87 4362	MN87 4572	MN87 4782	MN87 4992	MN87 5202	MN87 5412	MN87 5622	MN87 5832
170325	domestic duck	2017 Jan 12	40	Samadet	MN87 4363	MN87 4573	MN87 4783	MN87 4993	MN87 5203	MN87 5413	MN87 5623	MN87 5833
170330	domestic duck	2017 Jan 13	65	Antin	MN87 4364	MN87 4574	MN87 4784	MN87 4994	MN87 5204	MN87 5414	MN87 5624	MN87 5834
170331	domestic duck	2017 Jan 13	65	Antin	MN87 4365	MN87 4575	MN87 4785	MN87 4995	MN87 5205	MN87 5415	MN87 5625	MN87 5835
170338	domestic duck	2017 Jan 16	40	Samadet	MN87 4366	MN87 4576	MN87 4786	MN87 4996	MN87 5206	MN87 5416	MN87 5626	MN87 5836
170339	domestic duck	2017 Jan 13	32	Saint-Élix-Theux	MN87 4367	MN87 4577	MN87 4787	MN87 4997	MN87 5207	MN87 5417	MN87 5627	MN87 5837
170340	<i>Gallus Gallus</i>	2017 Jan 13	65	Coussan	MN87 4368	MN87 4578	MN87 4788	MN87 4998	MN87 5208	MN87 5418	MN87 5628	MN87 5838

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
170341	domestic duck	2017 Jan 13	40	Bahus-Soubiran	MN87 4369	MN87 4579	MN87 4789	MN87 4999	MN87 5209	MN87 5419	MN87 5629	MN87 5839
170349	domestic duck	2017 Jan 13	40	Samadet	MN87 4370	MN87 4580	MN87 4790	MN87 5000	MN87 5210	MN87 5420	MN87 5630	MN87 5840
170368	domestic duck	2017 Jan 16	40	Eugénie-les-Bains	MN87 4371	MN87 4581	MN87 4791	MN87 5001	MN87 5211	MN87 5421	MN87 5631	MN87 5841
170369	domestic duck	2017 Jan 14	40	Saint-Sever	MN87 4372	MN87 4582	MN87 4792	MN87 5002	MN87 5212	MN87 5422	MN87 5632	MN87 5842
170370	domestic duck	2017 Jan 14	65	Lubret-Saint-Luc	MN87 4373	MN87 4583	MN87 4793	MN87 5003	MN87 5213	MN87 5423	MN87 5633	MN87 5843
170406	<i>Buteo buteo</i>	2017 Jan 19	32	Barcugnan	MN87 4374	MN87 4584	MN87 4794	MN87 5004	MN87 5214	MN87 5424	MN87 5634	MN87 5844
170407	<i>Gallus Gallus</i>	2017 Jan 16	40	Samadet	MN87 4375	MN87 4585	MN87 4795	MN87 5005	MN87 5215	MN87 5425	MN87 5635	MN87 5845
170408	<i>Gallus Gallus</i>	2017 Jan 17	40	Aubagne	MN87 4376	MN87 4586	MN87 4796	MN87 5006	MN87 5216	MN87 5426	MN87 5636	MN87 5846
170409	<i>Numida meleagris</i>	2017 Jan 17	40	Pimbo	MN87 4377	MN87 4587	MN87 4797	MN87 5007	MN87 5217	MN87 5427	MN87 5637	MN87 5847
170432	domestic duck	2017 Jan 18	40	Pimbo	MN87 4378	MN87 4588	MN87 4798	MN87 5008	MN87 5218	MN87 5428	MN87 5638	MN87 5848
170433	<i>Gallus Gallus</i>	2017 Jan 16	40	Montgaillard	MN87 4379	MN87 4589	MN87 4799	MN87 5009	MN87 5219	MN87 5429	MN87 5639	MN87 5849
170436	<i>Gallus Gallus</i>	2017 Jan 17	40	Miramont-Sensacq	MN87 4380	MN87 4590	MN87 4800	MN87 5010	MN87 5220	MN87 5430	MN87 5640	MN87 5850
170437	domestic duck	2017 Jan 17	40	Bahus-Soubiran	MN87 4381	MN87 4591	MN87 4801	MN87 5011	MN87 5221	MN87 5431	MN87 5641	MN87 5851
170473	<i>Falco tinnunculus</i>	2017 Jan 21	32	Saint-Michel	MN87 4382	MN87 4592	MN87 4802	MN87 5012	MN87 5222	MN87 5432	MN87 5642	MN87 5852
170496	swan	2017 Feb 15	44	Vair-sur-Loire	MN87 4483	MN87 4693	MN87 4903	MN87 5113	MN87 5323	MN87 5533	MN87 5743	MN87 5953
170518	domestic duck	2017 Jan 18	64	Castetpugon	MN87 4383	MN87 4593	MN87 4803	MN87 5013	MN87 5223	MN87 5433	MN87 5643	MN87 5853
170612	domestic duck	2017 Jan 23	32	Lelin-Lapujolle	MN87 4384	MN87 4594	MN87 4804	MN87 5014	MN87 5224	MN87 5434	MN87 5644	MN87 5854
170683	domestic duck	2017 Jan 20	40	Benquet	MN87 4385	MN87 4595	MN87 4805	MN87 5015	MN87 5225	MN87 5435	MN87 5645	MN87 5855
170685	domestic duck	2017 Jan 23	65	Lapeyre	MN87 4386	MN87 4596	MN87 4806	MN87 5016	MN87 5226	MN87 5436	MN87 5646	MN87 5856
170686	<i>Gallus Gallus</i>	2017 Jan 24	40	Pimbo	MN87 4387	MN87 4597	MN87 4807	MN87 5017	MN87 5227	MN87 5437	MN87 5647	MN87 5857
170733	domestic duck	2017 Jan 25	32	Troncens	MN87 4388	MN87 4598	MN87 4808	MN87 5018	MN87 5228	MN87 5438	MN87 5648	MN87 5858
170734	domestic duck	2017 Jan 25	65	Sentous	MN87 4389	MN87 4599	MN87 4809	MN87 5019	MN87 5229	MN87 5439	MN87 5649	MN87 5859
170735	<i>Buteo buteo</i>	2017 Jan 25	32	Viella	MN87 4390	MN87 4600	MN87 4810	MN87 5020	MN87 5230	MN87 5440	MN87 5650	MN87 5860
170738	domestic duck	2017 Jan 24	65	Puydarrieux	MN87 4391	MN87 4601	MN87 4811	MN87 5021	MN87 5231	MN87 5441	MN87 5651	MN87 5861
170740	<i>Buteo buteo</i>	2017 Jan 27	32	Le Houga	MN87 4392	MN87 4602	MN87 4812	MN87 5022	MN87 5232	MN87 5442	MN87 5652	MN87 5862
170772	domestic duck	2017 Jan 27	32	Vergoignan	MN87 4393	MN87 4603	MN87 4813	MN87 5023	MN87 5233	MN87 5443	MN87 5653	MN87 5863
170773	<i>Gallus Gallus</i>	2017 Jan 27	40	Mant	MN87 4394	MN87 4604	MN87 4814	MN87 5024	MN87 5234	MN87 5444	MN87 5654	MN87 5864
170774	<i>Gallus Gallus</i>	2017 Jan 24	40	Latrille	MN87 4395	MN87 4605	MN87 4815	MN87 5025	MN87 5235	MN87 5445	MN87 5655	MN87 5865
170775	domestic duck	2017 Jan 24	64	Arzacq-Arraziguët	MN87 4396	MN87 4606	MN87 4816	MN87 5026	MN87 5236	MN87 5446	MN87 5656	MN87 5866
170806	domestic duck	2017 Jan 25	32	Tarsac	MN87 4397	MN87 4607	MN87 4817	MN87 5027	MN87 5237	MN87 5447	MN87 5657	MN87 5867
170820	domestic duck	2017 Jan 30	40	Préchacq-les-Bains	MN87 4398	MN87 4608	MN87 4818	MN87 5028	MN87 5238	MN87 5448	MN87 5658	MN87 5868
170822	domestic duck	2017 Jan 30	64	Carrère	MN87 4399	MN87 4609	MN87 4819	MN87 5029	MN87 5239	MN87 5449	MN87 5659	MN87 5869
170974	domestic duck	2017 Jan 31	65	Puydarrieux	MN87 4400	MN87 4610	MN87 4820	MN87 5030	MN87 5240	MN87 5450	MN87 5660	MN87 5870
170975	domestic duck	2017 Jan 31	65	Puydarrieux	MN87 4401	MN87 4611	MN87 4821	MN87 5031	MN87 5241	MN87 5451	MN87 5661	MN87 5871

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
170976	domestic goose	2017 Feb 1	65	Lalanne-Trie	MN87 4402	MN87 4612	MN87 4822	MN87 5032	MN87 5242	MN87 5452	MN87 5662	MN87 5872
170977	unknown	2017 Jan 27	32	Labéjan	MN87 4403	MN87 4613	MN87 4823	MN87 5033	MN87 5243	MN87 5453	MN87 5663	MN87 5873
171131	domestic duck	2017 Feb 2	40	Hinx	MN87 4404	MN87 4614	MN87 4824	MN87 5034	MN87 5244	MN87 5454	MN87 5664	MN87 5874
171133	domestic duck	2017 Feb 3	40	Goos	MN87 4405	MN87 4615	MN87 4825	MN87 5035	MN87 5245	MN87 5455	MN87 5665	MN87 5875
171134	domestic duck	2017 Feb 3	64	Miossens-Lanusse	MN87 4406	MN87 4616	MN87 4826	MN87 5036	MN87 5246	MN87 5456	MN87 5666	MN87 5876
171202	domestic duck	2017 Feb 3	40	Toulouze	MN87 4407	MN87 4617	MN87 4827	MN87 5037	MN87 5247	MN87 5457	MN87 5667	MN87 5877
171253	domestic duck	2017 Feb 5	40	Gamarde-les-Bains	MN87 4408	MN87 4618	MN87 4828	MN87 5038	MN87 5248	MN87 5458	MN87 5668	MN87 5878
171265	swan	2017 Feb 20	1	Versailleux	MN87 4480	MN87 4690	MN87 4900	MN87 5110	MN87 5320	MN87 5530	MN87 5740	MN87 5950
171267	swan	2017 Feb 21	1	Marlieux	MN87 4490	MN87 4700	MN87 4910	MN87 5120	MN87 5330	MN87 5540	MN87 5750	MN87 5960
171270	domestic duck	2017 Feb 7	40	Gibret	MN87 4409	MN87 4619	MN87 4829	MN87 5039	MN87 5249	MN87 5459	MN87 5669	MN87 5879
171275	domestic duck	2017 Feb 7	32	Fustérouau	MN87 4410	MN87 4620	MN87 4830	MN87 5040	MN87 5250	MN87 5460	MN87 5670	MN87 5880
171278	domestic goose	2017 Feb 6	40	Montaut	MN87 4411	MN87 4621	MN87 4831	MN87 5041	MN87 5251	MN87 5461	MN87 5671	MN87 5881
171376	swan	2017 Mar 25	88	Sanchey	MN87 4489	MN87 4699	MN87 4909	MN87 5119	MN87 5329	MN87 5539	MN87 5749	MN87 5959
171377	swan	2017 Mar 26	1	Versailleux	MN87 4482	MN87 4692	MN87 4902	MN87 5112	MN87 5322	MN87 5532	MN87 5742	MN87 5952
171378	domestic duck	2017 Feb 7	40	Gibret	MN87 4412	MN87 4622	MN87 4832	MN87 5042	MN87 5252	MN87 5462	MN87 5672	MN87 5882
171379	domestic duck	2017 Feb 6	65	Sadournin	MN87 4413	MN87 4623	MN87 4833	MN87 5043	MN87 5253	MN87 5463	MN87 5673	MN87 5883
171408	domestic duck	2017 Feb 7	40	Baigts	MN87 4414	MN87 4624	MN87 4834	MN87 5044	MN87 5254	MN87 5464	MN87 5674	MN87 5884
171410	domestic duck	2017 Feb 8	40	Cassen	MN87 4415	MN87 4625	MN87 4835	MN87 5045	MN87 5255	MN87 5465	MN87 5675	MN87 5885
171413	<i>Buteo buteo</i>	2017 Feb 8	32	Belloc-Saint-Clamens	MN87 4416	MN87 4626	MN87 4836	MN87 5046	MN87 5256	MN87 5466	MN87 5676	MN87 5886
171455	domestic duck	2017 Feb 11	40	Cassen	MN87 4417	MN87 4627	MN87 4837	MN87 5047	MN87 5257	MN87 5467	MN87 5677	MN87 5887
171457	domestic duck	2017 Feb 11	40	Sort-en-Chalosse	MN87 4418	MN87 4628	MN87 4838	MN87 5048	MN87 5258	MN87 5468	MN87 5678	MN87 5888
171461	domestic duck	2017 Feb 12	40	gamarde	MN87 4419	MN87 4629	MN87 4839	MN87 5049	MN87 5259	MN87 5469	MN87 5679	MN87 5889
171462	domestic duck	2017 Feb 12	40	Habas	MN87 4420	MN87 4630	MN87 4840	MN87 5050	MN87 5260	MN87 5470	MN87 5680	MN87 5890
171463	domestic duck	2017 Feb 12	40	Toulouze	MN87 4421	MN87 4631	MN87 4841	MN87 5051	MN87 5261	MN87 5471	MN87 5681	MN87 5891
171466	domestic duck	2017 Feb 10	32	Aignan	MN87 4422	MN87 4632	MN87 4842	MN87 5052	MN87 5262	MN87 5472	MN87 5682	MN87 5892
171467	domestic duck	2017 Feb 10	40	Poyartin	MN87 4423	MN87 4633	MN87 4843	MN87 5053	MN87 5263	MN87 5473	MN87 5683	MN87 5893
171468	domestic duck	2017 Feb 10	40	Poyartin	MN87 4424	MN87 4634	MN87 4844	MN87 5054	MN87 5264	MN87 5474	MN87 5684	MN87 5894
171469	domestic duck	2017 Feb 11	40	Hinx	MN87 4425	MN87 4635	MN87 4845	MN87 5055	MN87 5265	MN87 5475	MN87 5685	MN87 5895
171471	<i>Gallus Gallus</i>	2017 Feb 11	40	Narrosse	MN87 4426	MN87 4636	MN87 4846	MN87 5056	MN87 5266	MN87 5476	MN87 5686	MN87 5896
171472	domestic duck	2017 Feb 11	40	Montaut	MN87 4427	MN87 4637	MN87 4847	MN87 5057	MN87 5267	MN87 5477	MN87 5687	MN87 5897
171486	domestic duck	2017 Feb 11	40	Souprosse	MN87 4428	MN87 4638	MN87 4848	MN87 5058	MN87 5268	MN87 5478	MN87 5688	MN87 5898
171514	domestic duck	2017 Feb 11	40	Hinx	MN87 4429	MN87 4639	MN87 4849	MN87 5059	MN87 5269	MN87 5479	MN87 5689	MN87 5899
171525	<i>Gallus Gallus</i>	2017 Feb 13	40	Audignon	MN87 4430	MN87 4640	MN87 4850	MN87 5060	MN87 5270	MN87 5480	MN87 5690	MN87 5900
171533	domestic duck	2017 Feb 13	40	Souprosse	MN87 4431	MN87 4641	MN87 4851	MN87 5061	MN87 5271	MN87 5481	MN87 5691	MN87 5901

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
171534	<i>Gallus</i>	2017 Feb	40	Magescq	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
	<i>Gallus</i>	12			4432	4642	4852	5062	5272	5482	5692	5902
171535	domestic duck	2017 Feb	40	Candresse	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		12			4433	4643	4853	5063	5273	5483	5693	5903
171536	domestic duck	2017 Feb	40	Sort-en-Chalosse	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4434	4644	4854	5064	5274	5484	5694	5904
171537	<i>Gallus</i>	2017 Feb	40	Laglorieuse	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
	<i>Gallus</i>	14			4435	4645	4855	5065	5275	5485	5695	5905
171539	domestic duck	2017 Feb	40	Caupenne	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4436	4646	4856	5066	5276	5486	5696	5906
171541	domestic duck	2017 Feb	40	Caupenne	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4437	4647	4857	5067	5277	5487	5697	5907
171542	domestic duck	2017 Feb	40	Tilh	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		13			4438	4648	4858	5068	5278	5488	5698	5908
171543	domestic duck	2017 Feb	40	Bastennes	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4439	4649	4859	5069	5279	5489	5699	5909
171546	domestic duck	2017 Feb	40	Habas	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4440	4650	4860	5070	5280	5490	5700	5910
171550	domestic duck	2017 Feb	40	Souprosse	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4441	4651	4861	5071	5281	5491	5701	5911
171585	swan	2017 Apr	1	Marlieux	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		27			4488	4698	4908	5118	5328	5538	5748	5958
171599	unknown	2017 Feb	40	Montaut	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		15			4442	4652	4862	5072	5282	5492	5702	5912
171603	domestic duck	2017 Feb	40	Saunac-et-Cambran	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		16			4443	4653	4863	5073	5283	5493	5703	5913
171606	unknown	2017 Feb	40	Toulouzette	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		16			4444	4654	4864	5074	5284	5494	5704	5914
171607	domestic duck	2017 Feb	40	Baigts	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		15			4445	4655	4865	5075	5285	5495	5705	5915
171639	domestic duck	2017 Feb	40	Nousse	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		15			4446	4656	4866	5076	5286	5496	5706	5916
171641	domestic duck	2017 Feb	40	Samadet	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		14			4447	4657	4867	5077	5287	5497	5707	5917
171650	domestic duck	2017 Feb	40	Maylis	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		15			4448	4658	4868	5078	5288	5498	5708	5918
171779	domestic duck	2017 Feb	47	Cancon	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		19			4484	4694	4904	5114	5324	5534	5744	5954
171860	domestic duck	2017 Feb	40	Saunac-et-Cambran	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		21			4449	4659	4869	5079	5289	5499	5709	5919
171874	domestic duck	2017 Feb	40	Castelsarrasin	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		22			4450	4660	4870	5080	5290	5500	5710	5920
171884	domestic duck	2017 Feb	40	Lamothe	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		20			4451	4661	4871	5081	5291	5501	5711	5921
171915	<i>Gallus</i>	2017 Feb	40	Donzacq	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
	<i>Gallus</i>	21			4452	4662	4872	5082	5292	5502	5712	5922
171928	domestic duck	2017 Mar	40	Gaujacq	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		2			4453	4663	4873	5083	5293	5503	5713	5923
171929	domestic duck	2017 Feb	40	Gaujacq	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		24			4454	4664	4874	5084	5294	5504	5714	5924
171932	domestic duck	2017 Feb	40	Saint-Saturnin	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		24			4455	4665	4875	5085	5295	5505	5715	5925
171953	domestic goose	2017 Jun	69	Saint-Étienne-des-Oullières	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		20			4487	4697	4907	5117	5327	5537	5747	5957
171960	swan	2017 Jun	88	Archettes	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		23			4485	4695	4905	5115	5325	5535	5745	5955
172002	<i>Gallus</i>	2017 Feb	40	Misson	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
	<i>Gallus</i>	25			4456	4666	4876	5086	5296	5506	5716	5926
172003	<i>Gallus</i>	2017 Feb	40	Hauriet	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
	<i>Gallus</i>	25			4457	4667	4877	5087	5297	5507	5717	5927
172004	domestic duck	2017 Feb	40	Peyrehorade	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		25			4458	4668	4878	5088	5298	5508	5718	5928
172012	domestic duck	2017 Feb	40	Bénesse-Mareme	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		23			4459	4669	4879	5089	5299	5509	5719	5929
172038	domestic duck	2017 Feb	47	Villefranche-du-Queyran	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		27			4460	4670	4880	5090	5300	5510	5720	5930
172057	domestic duck	2017 Feb	64	Moncayolle	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
		27			4461	4671	4881	5091	5301	5511	5721	5931

Sample no.	Host	Collection date	Dept no.	Location	Gene segment							
					PB2	PB1	PA	HA	NP	NA	M	NS
172092	<i>Gallus</i>	2017 Feb 28	40	Gaujacq	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172093	<i>Gallus</i>	2017 Feb 28	40	Doazit	4462	4672	4882	5092	5302	5512	5722	5932
172094	domestic duck	2017 Mar 1	40	Saint-Jean-de-Marsacq	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172096	domestic duck	2017 Mar 1	64	Bugnein	4464	4674	4884	5094	5304	5514	5724	5934
172137	<i>Gallus</i>	2017 Mar 2	40	Doazit	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172144	domestic duck	2017 Feb 27	40	Saint-Étienne-d'Orthe	4466	4676	4886	5096	5306	5516	5726	5936
172182	domestic duck	2017 Mar 1	40	Saint-Lon-les-Mines	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172287	<i>Gallus</i>	2017 Mar 7	64	Bidache	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172289	domestic duck	2017 Mar 4	64	Came	4469	4679	4889	5099	5309	5519	5729	5939
172331	domestic duck	2017 Mar 13	64	Oraàs	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172362	domestic duck	2017 Mar 18	64	Saint-Gladie-Arrive-Munein	4471	4681	4891	5101	5311	5521	5731	5941
172383	<i>Gallus</i>	2017 Mar 21	64	Saint-Gladie-Arrive-Munein	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172384	domestic duck	2017 Mar 22	64	Monfort	4473	4683	4893	5103	5313	5523	5733	5943
172390	domestic duck	2017 Mar 21	64	Castelnau	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
172394	domestic duck	2017 Mar 21	64	Préchacq-Navarrenx	4474	4684	4894	5104	5314	5524	5734	5944
172610	<i>Gallus</i>	2017 Jun 26	59	Grillon	MN87	MN87	MN87	MN87	MN87	MN87	MN87	MN87
	<i>Gallus</i>				4477	4687	4897	5107	5317	5527	5737	5947

*Accession nos. for samples 161104,161108 from GISAID. Accession nos. of remaining samples from Genbank. Dept, department; HA, hemagglutinin; M, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase acidic protein; PB1, polymerase basic 1 protein; PB2, polymerase basic 2 protein.

†Most domestic ducks used for foie gras production are a hybrid of Muscovy (*Cairina moschata*) and Pekin ducks.

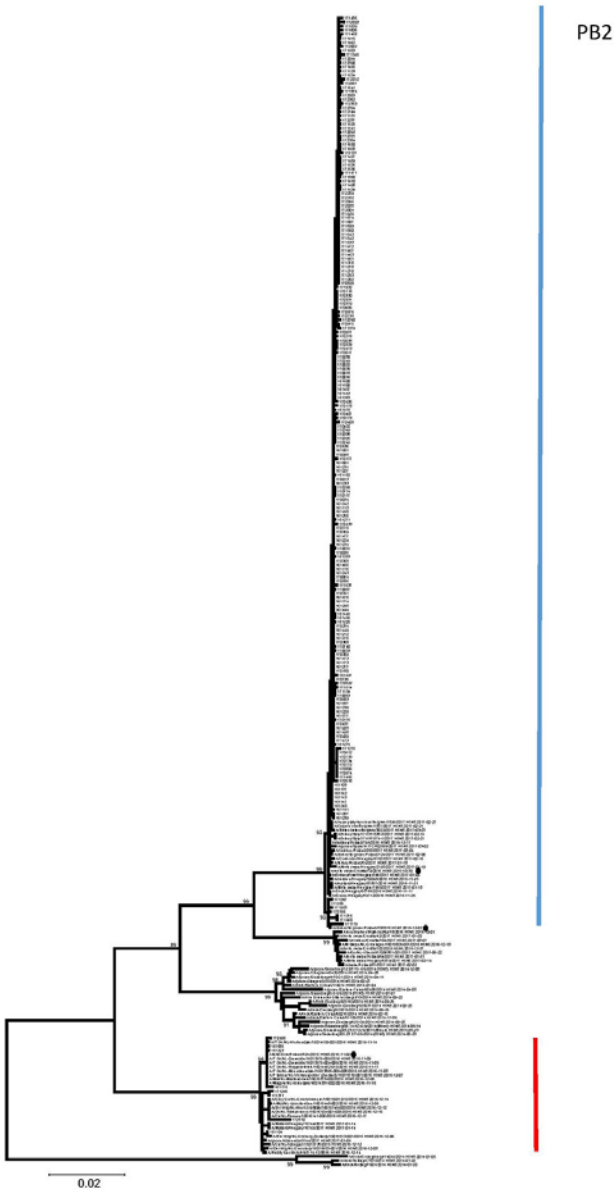
Appendix Table 2. Highly pathogenic influenza A(H5N8) virus from wild and domestic birds, France*

Species	Detected			Sequenced					
	Setting		Total	Setting		Genotype			Total
	Southwestern France	Other		Southwestern France	Other	A	B	C	
Wild birds									
Gull	1	2	3	0	1	0	1	0	1
Magpie	1	0	1	0	0	NA	NA	NA	0
Pigeon/dove	4	0	4	0	0	NA	NA	NA	0
Eurasian wigeon	0	1	1	0	1	0	1	0	1
Swan	0	25	25	0	8	0	3	5	8
Common buzzard	7	0	7	4	0	4	0	0	4
Kestrel	1	0	1	1	0	1	0	0	1
Heron/egret	2	3	5	0	0	NA	NA	NA	0
Song thrush	1	0	1	0	0	NA	NA	NA	0
Goose	0	3	3	0	0	NA	NA	NA	0
Subtotal	17	34	51	5	10	5	5	5	15
Domestic and captive birds									
Duck	388	3	391	164	0	163	0	1	164
Chicken	52	2	54	22	2	22	2	0	24
Goose	4	2	6	2	1	2	0	1	3
Guinea fowl	2	0	2	1	0	1	0	0	1
Turkey	0	1	1	1	0	1	0	0	1
Unknown/other/multispecies	33	1	34	3	1	3	1	0	4
Subtotal	479	9	488	193	4	192	3	2	197

*NA, not applicable.

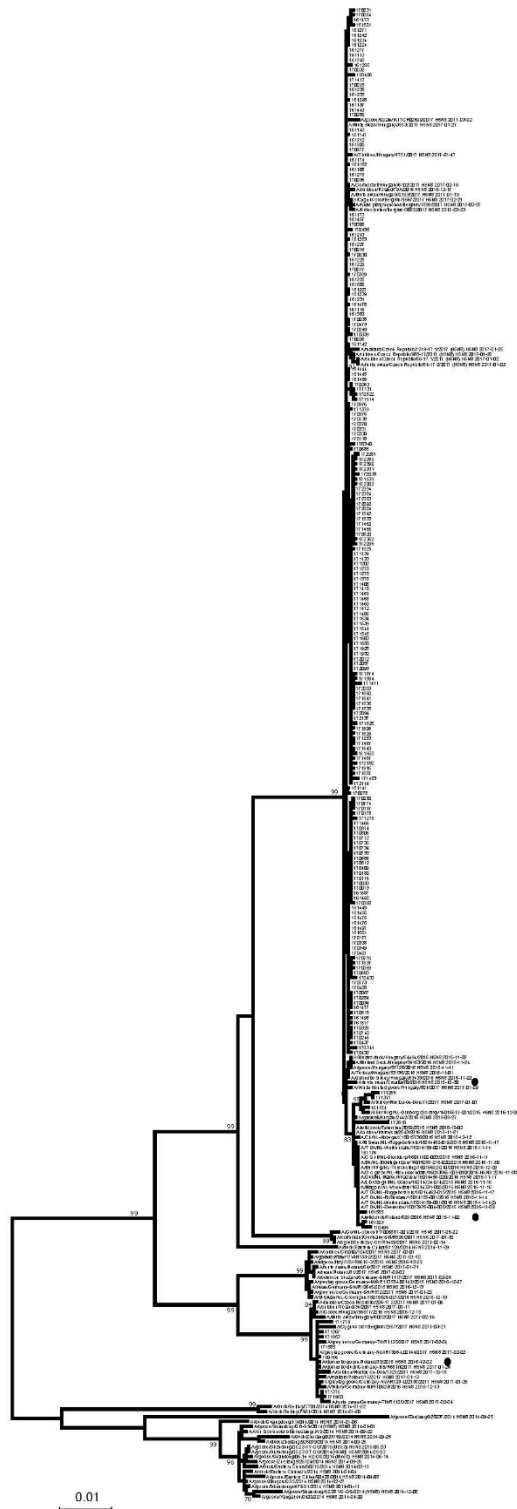
Appendix Table 3. Most recent common ancestor of selected highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17

Geocluster	Estimated time of most recent common ancestor	95% Highest probability density interval	
		Start	End
1	2016 Nov 16	2016 Nov 9	2016 Nov 23
5	2017 Jan 15	2017 Jan 7	2017 Jan 23

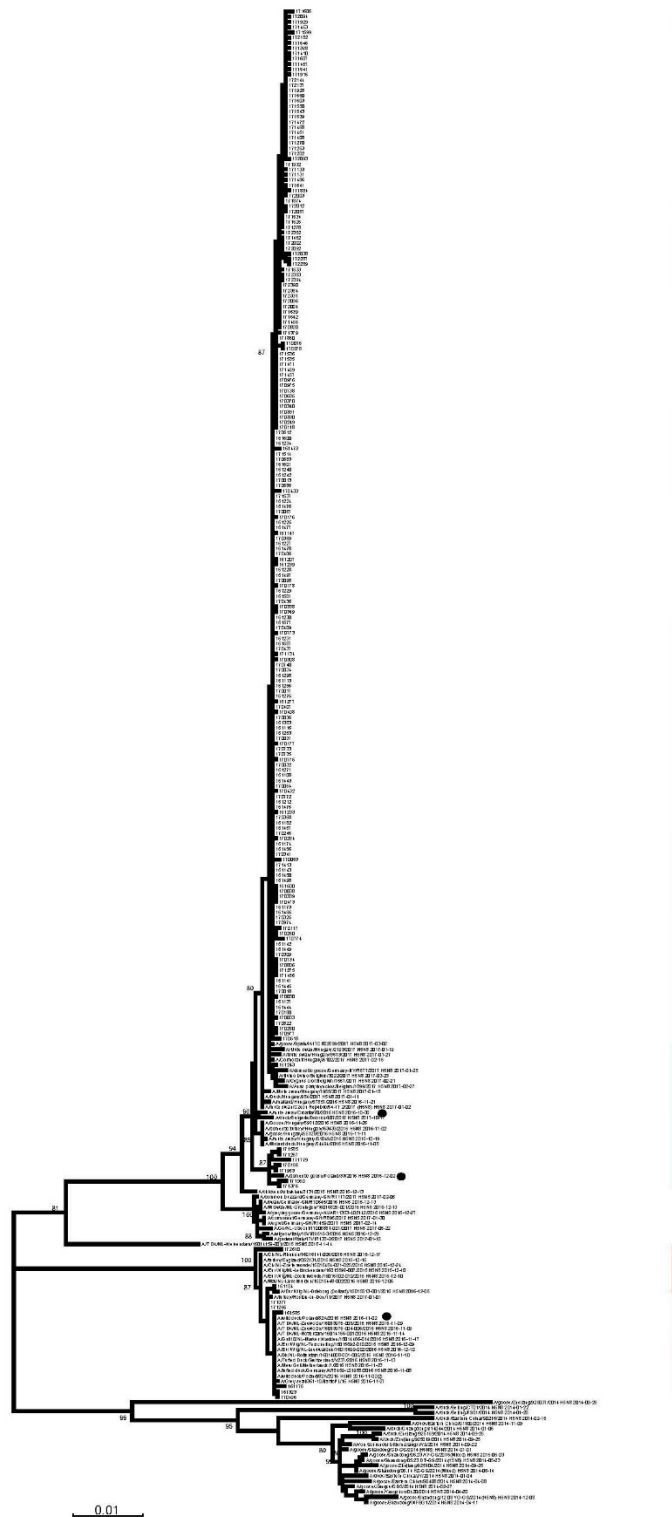


Appendix Figure 1. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on polymerase basic 2 gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.

PB1



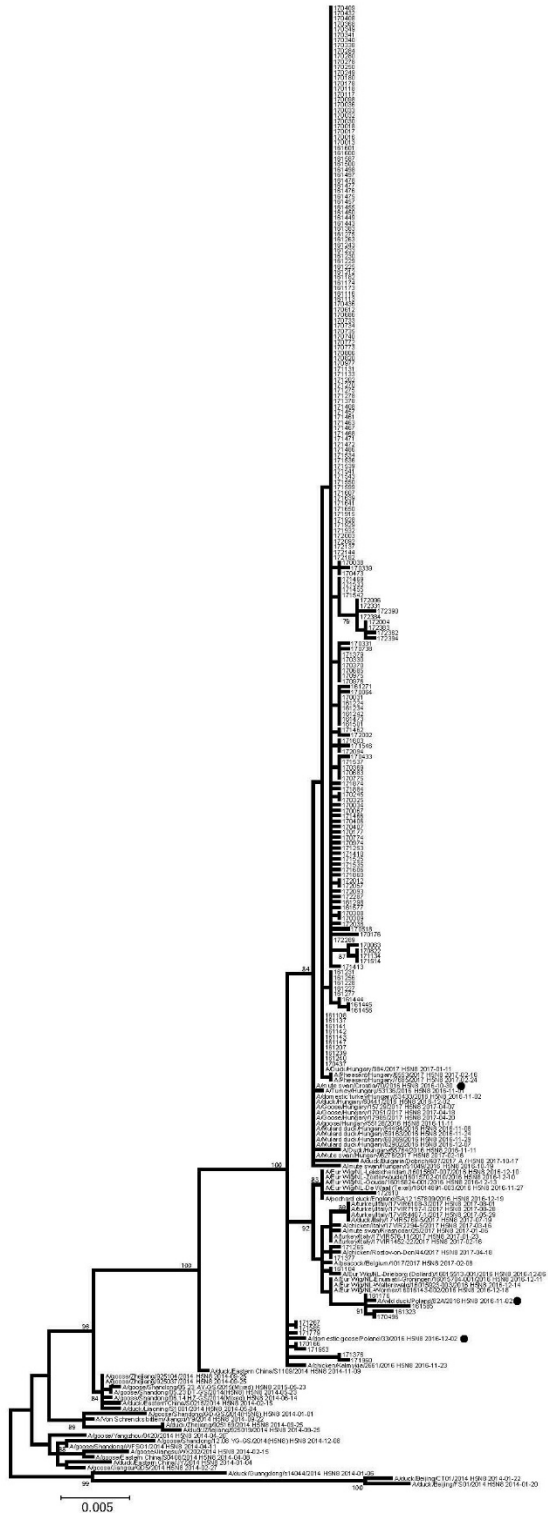
Appendix Figure 2. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on polymerase basic 1 gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.



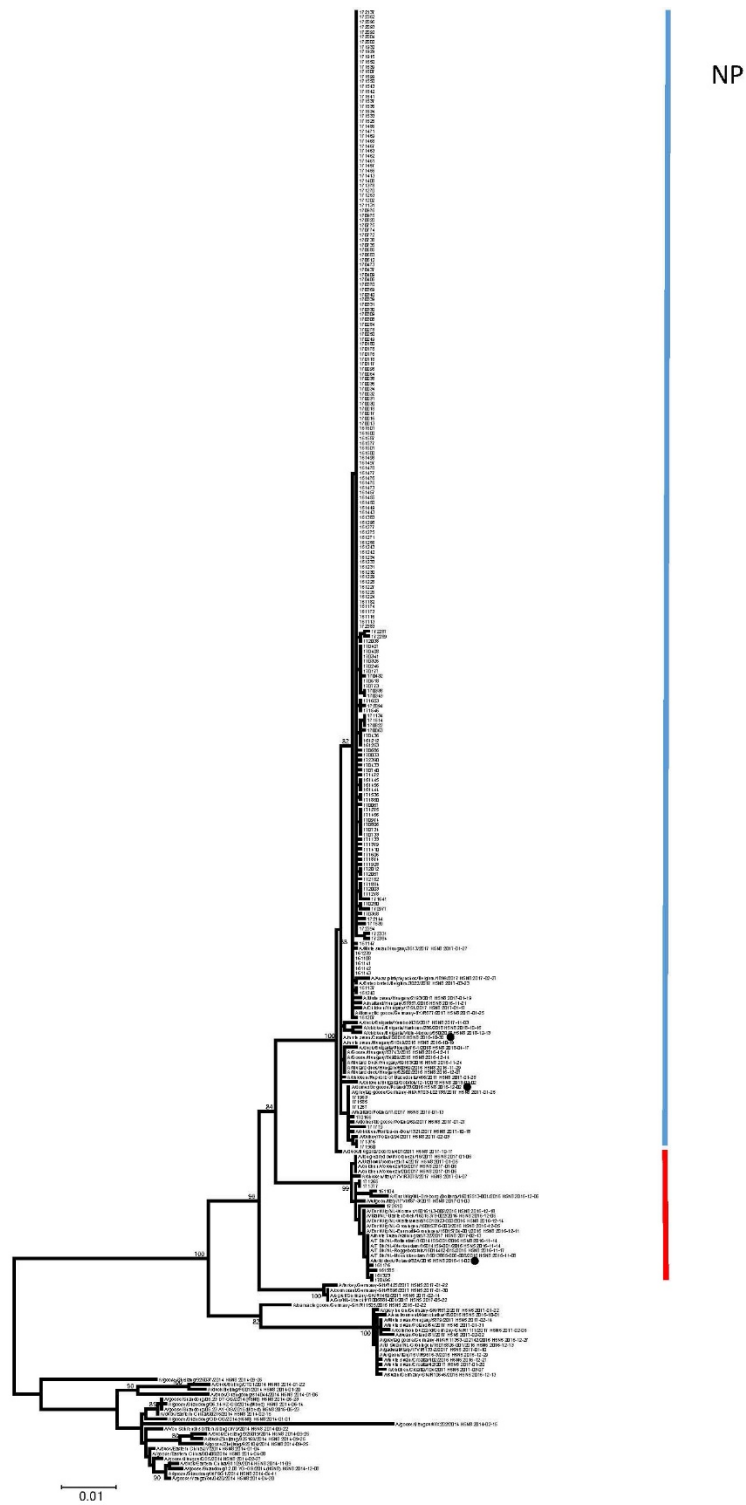
PA

Appendix Figure 3. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on polymerase acidic protein (PA) gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.

HA

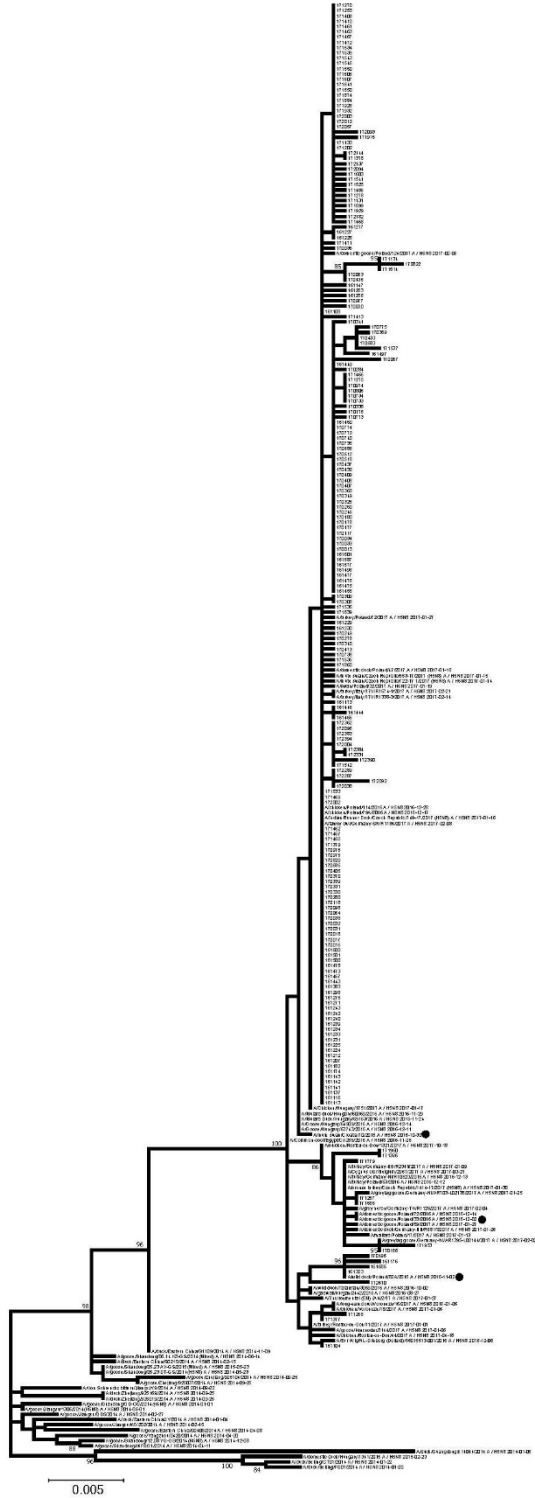


Appendix Figure 4. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on hemagglutinin gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.

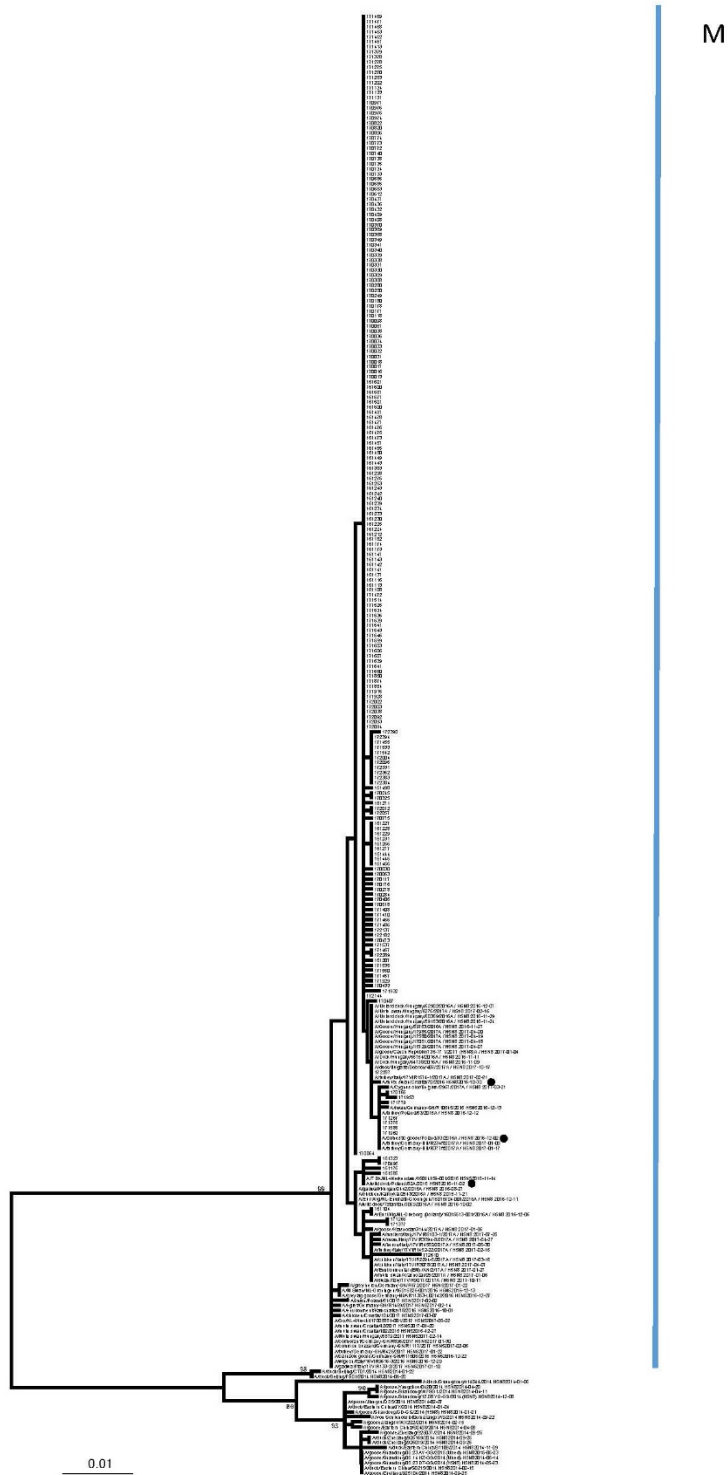


Appendix Figure 5. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on nucleoprotein (NP) gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.

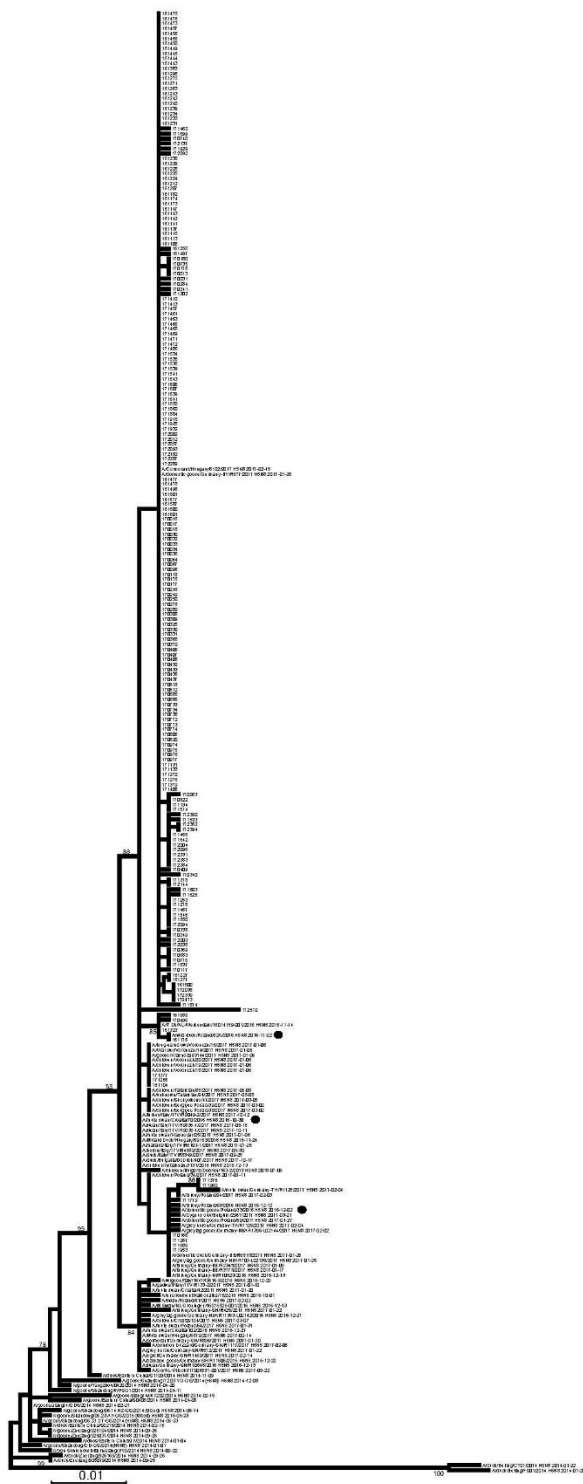
NA



Appendix Figure 6. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on neuraminidase gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.



Appendix Figure 7. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on matrix protein (M) gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.



NS

Appendix Figure 8. Maximum-likelihood phylogenetic tree of highly pathogenic avian influenza H5N8 genotype A viruses, France, 2016–17. Phylogeny is based on nonstructural protein (NS) gene segment. Bootstrap values >75 indicated. Colors indicate cluster. Black dots indicate reference sequences. Scale is nucleotide substitutions/site.