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SHORT COMMUNICATION

Multiple independent introductions of highly pathogenic avian influenza H5 viruses during the 2020–2021 epizootic in France

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Abstract

During winter 2020–2021, France and other European countries were severely affected by highly pathogenic avian influenza H5 viruses of the Gs/GD/96 lineage, clade 2.3.4.4b. In total, 519 cases occurred, mainly in domestic waterfowl farms in Southwestern France. Analysis of viral genomic sequences indicated that 3 subtypes of HPAI H5 viruses were detected (H5N1, H5N3, H5N8), but most French viruses belonged to the H5N8 subtype genotype A, as Europe. Phylogenetic analyses of HPAI H5N8 viruses revealed that the French sequences were distributed in 9 genogroups, suggesting 9 independent introductions of H5N8 from wild birds, in addition to the 2 introductions of H5N1 and H5N3.

KEYWORDS

birds, epizootic, highly pathogenic influenza A virus, H5N8 subtype

1 | INTRODUCTION

Since August 2020, Russia, Kazakhstan and Europe have been confronted once again with an epizootic of highly pathogenic avian influenza (HPAI) caused by H5 viruses. In Europe during the season 2020–2021, at least 5 subtypes of HPAI viruses (H5N8, H5N1, H5N3, H5N4 and H5N5) have been identified, that can be subdivided into 19 genotypes; the H5N8 subtype was the most represented by the genotype A (European Food Safety et al., 2022; Fusaro, 2021). All

these subtypes have an HA segment belonging to clade 2.3.4.4b of the A/goose/Guangdong/1/1996 lineage. From September 2020 to the end of March 2021, more than 1800 cases in wild birds and 800 outbreaks in poultry have been reported in Europe. Many HPAI viruses were detected in France during season 2020–2021 ($n = 519$), among 492 were from poultry farms. The majority of poultry detections were from duck farms in Southwestern France. The sequencing of the complete virus genomes from different outbreaks in poultry and cases in wild birds in France allowed to study the viral diversity observed

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and, after comparison with other sequences available at the international level, to deduce the potential independent introductions into the French territory.

2 | MATERIALS AND METHODS

From November 2020 to July 2021, tracheal and cloacal swabs were taken for each suspected case of HPAI in wild or domestic birds. Viral RNA was extracted from mixtures of up to 5 swab supernatants (RNeasy mini kit, Qiagen). These RNAs were then tested by real-time RT-PCR (rRT-PCR) for the M gene and rRT-PCR for the H5 gene by approved departmental laboratories (Slomka et al., 2007). The positive samples were then transferred to the National Reference Laboratory for Avian Influenza (LNR IA, Anses-Ploufragan-Plouzané-Niort) for specific rRT-PCR of the H5 gene of clade 2.3.4.4b (Naguib et al., 2017) and the N8 gene (Hoffmann et al., 2016) ($n = 1084$). The nucleotide sequence framing the haemagglutinin cleavage site was also determined by end-point RT-PCR of the H5 Kha1/Kha3 gene fragment and sequencing (Slomka et al., 2007) to confirm the pathotype (sequencing of the haemagglutinin cleavage site) ($n = 162$). Each French outbreak site was selected to obtain a full genome viral sequence, except for outbreaks from Southwestern France poultry farms where only the 11 first detected outbreak sites were selected. Indeed all sequences from Southwestern France poultry outbreaks belong to the same genotype and formed a monophyletic cluster (data not shown). For each selected French outbreak site, only one sample was used for full genome sequencing ($n = 53$). The 8 viral segments were amplified by RT-PCR (Zhou et al., 2009). After preparation of the libraries, the amplicons were sequenced on an Ion Proton sequencer (life technologies) (Briand et al., 2021). Only samples with almost fully sequenced genomic segments ($>95\%$ of complete genome length available) ($n = 45/53$; Table 1) were used for the following analyses and were deposited to Genbank (Table S1). Each segment was compared to available sequences in the GISAID database (week 09, 2022). All full genome sequences of the HPAI H5N8 genotype A collected from September 2020 to August 2021 in Europe and Kazakhstan were selected from GISAID (Table S3). In addition, the viral genome of the A/chicken/Iraq/1/2020 strain was added in the analyses as prototype of the genotype A HPAI H5N8. In parallel, the individual eight viral segments and their concatenated counterpart were aligned with MAFFT. The Neighbor-Joining method based on the Tamura-3-parameter model was performed on the alignment to obtain phylogenetic trees with 1000 bootstrap replicates using the MEGA7 software (Kumar et al., 2016). A preliminary verification that no reassortant HPAI H5N8 viruses were present was undertaken using the RDP4 software in order to validate the phylogenetic analysis of concatenated genome sequences.

3 | RESULTS

November 10, week 46, 2020, clinical signs evocative of highly pathogenic avian influenza (HPAI) were observed among birds in a pet

store in Northern Corsica n°2B French district (Figure 1). The presence of HPAI H5N8 virus was confirmed by the French national reference laboratory (NRL) for avian influenza (Ploufragan, France). Following this confirmation, ornamental bird flocks with direct or indirect epidemiological links with this outbreak were investigated. In this regard, 11 HPAI H5N8 positive outbreak sites were detected, 9 of which were located on the island of Corsica (district n°2A and n°2B), and two additional on mainland France, 1 from each French districts n°78 and 59. For the outbreak sites in district n°78 and n°59, samples were collected from birds in a petshop and from the environment in the establishment of a geese supplier, respectively. The epidemiological investigations realized about these 11 outbreaks seems to indicate that the geese supplier from district n°59 was the index case, and that the viral introduction in the geese farm was from wild birds. Subsequently, the spread of the virus was due to a domestic bird transport. As a consequence of the detection of several HPAI H5 outbreaks in France, the risk level for introduction of HPAI in France was raised to 'high' and passive surveillance in wild birds was strengthened (week 47, 2020; French Ministry of Agriculture and Food, 2020). Subsequently, HPAI H5 viruses were also detected in France in wild and captive birds, and massively in poultry, notably affecting the two major domestic duck production areas in western France (Agence nationale de sécurité sanitaire, 2017) (Figure 2 dashed circles). During the epizootic period, the number of HPAI H5 cases detected per week in France was variable (Figure 1). From weeks 46 to 51 of 2020, between 2 and 5 confirmations of HPAI H5 per week were detected, mainly from non-commercial poultry holdings and from wild birds. Thereafter, despite the control measures implemented the number of cases per week dramatically increased to reach a maximum of 147 confirmed cases in week 2 of 2021, almost only in Southwestern France (Figure 1). This sudden increase was due to the introduction of the virus in a high-density duck farming area of Southwestern France where significant numbers of domestic ducks were still kept outdoors although a compulsory housing order of all poultry was mandatory by French regulations in periods of high HPAI risk (week 46, 2020) (Agence nationale de sécurité sanitaire, 2021). After massive stamping out in this area, in week 6 of 2021, the number of cases decreased to less than 13 HPAI H5 cases per week. The last detection of HPAI H5 occurred in France in July 2, week 26, 2021, in captive birds (Figure 1), 9 weeks after the penultimate HPAI virus detection. In total, from week 46, 2020 to week 26, 2021, 519 cases of HP H5 were detected in France: 24 from wild birds, 2 from captive birds, 492 from poultry (475 were located in Southwestern France) and one from environmental samples (Table 1). Of the 519 cases, 465 were identified as HPAI H5N8 viruses, three were identified as HPAI H5N3 from red knots and Eurasian curlews and one as HPAI H5N1 from a brant goose (*Branta bernicla*) (Table S1; Figure 2). Additionally, 50 HPAI H5 viruses remained untyped from farms located in Southwestern France, probably due to the low viral genome load in these samples.

All French H5N8 genomes belonged to the genotype A with HA segment from clade 2.3.4.4b, corresponding to the most frequently detected genotype among the seven H5N8 HPAI virus genotypes described in Europe last winter (Fusaro, 2021). The eight concatenated

TABLE 1 Number of HPAI H5 cases detected in French districts according to the sampled bird category

	French district	Bird category			Environment
		Wild bird	Captive bird	Poultry	
Rest of France	Corse du sud – 2A	0	0	1 (1)	0
	Haute-Corse – 2B	2	0	6 (5)	0
	Allier – 03	1 (1)	0	0	0
	Ardennes – 08	3 (1)	0	2 (2)	0
	Bouches-du-Rhones – 13	1 (1)	0	0	0
	Calvados – 14	1 (1)	0	0	0
	Loire – 42	3 (3)	0	0	0
	Loire-Atlantique – 44	1 (1)	0	0	0
	Loiret – 45	0	1 (1)	0	0
	Manche – 50	4 (4)	0	0	0
	Meurthe-et-Moselle – 54	2 (2)	0	0	0
	Morbihan – 56	2	0	0	0
	Nord – 59	0	0	0	1
	Bas-Rhin – 67	2 (2)	1 (1)	1 (1)	0
	Haut-Rhin – 68	1 (1)	0	1 (1)	0
	Haute-Savoie – 74	0	0	1 (1)	0
	Yvelines – 78	0	0	1	0
	Deux-Sèvres – 79	0	0	1 (1)	0
	Vendée – 85	0	0	3 (3)	0
South western France	Haute-Garonne – 31	0	0	1	0
	Gers – 32	0	0	66	0
	Landes – 40	1	0	341 (9)	0
	Lot-et-Garonne – 47	0	0	2	0
	Pyrénées-Atlantique – 64	0	0	58	0
	Hautes-Pyrénées – 65	0	0	7 (2)	0
		24 (17)	2(2)	492 (26)	1

Note: In brackets, the numbers of full genomes obtained and used for this study.

viral segments of French HPAI H5N8 were compared to the full viral genome available at March 2022 on GISAID as described above. As the phylogenetic analyses of individual AI segment, phylogenetic analysis of concatenated genomes was performed suggesting that at least nine independent introductions in France of HPAI H5N8 viruses occurred, represented by nine identified French genogroups (Figure S1). Genogroup 1 was the most important by the number of detections and geographical spread. Indeed, it was responsible for almost all infection cases detected before week 2, 2021 and was detected over a large geographical area including French districts 2A, 2B, 13, 14, 40, 44, 54, 65, 79 and 85 (Figure 2). The genomes of this genogroup were closely related to the viruses detected in England, Belgium, Ireland and the Netherlands at the end of 2020 (Figure S1). In addition, among all first sequences obtained from Southwestern France, at the exception of a sequence (21P000799; Figure S1) detected from captive black swan belonging to genotype 2, viral sequences belonged to genogroup 1 and constituted a monophyletic cluster and were very closely related suggesting farm-to-farm transmission.

All other phylogenetic genogroups were only sporadically detected in France and identified from backyard, captive birds or wild birds. For each genogroups their detections were either unique or several times in close geographical areas and on a reduced timespan, excepted for genogroup 5, which were identified in Northern France (district n°08 in week 12, 2021) and in the centre of France (district n°45 in week 26, 2021), over 3 months later. This result suggested a possible undetected circulation of virus belonging to this genogroup 5 in France during few months possibly in commensal wild birds or due to persistence of infectious virus in the environment (Ramey et al., 2022).

In addition to HPAI H5N8 subtype, HPAI H5N1 and H5N3 was detected in wild birds in district n°50 on week 11, 2021 and on week 3, 2021, respectively. The HPAI H5N1 and H5N3 viruses exhibited a segment constellation similar to those already described in Italy, Scotland and Hungary, or in Germany, Ireland and Denmark, respectively (Table S2) (Fusaro, 2021).

In France, the HPAI H5 epizootic in 2020–2021 was very similar to the previous HPAI H5 epizootic in 2016–2017 (Briand et al., 2021).

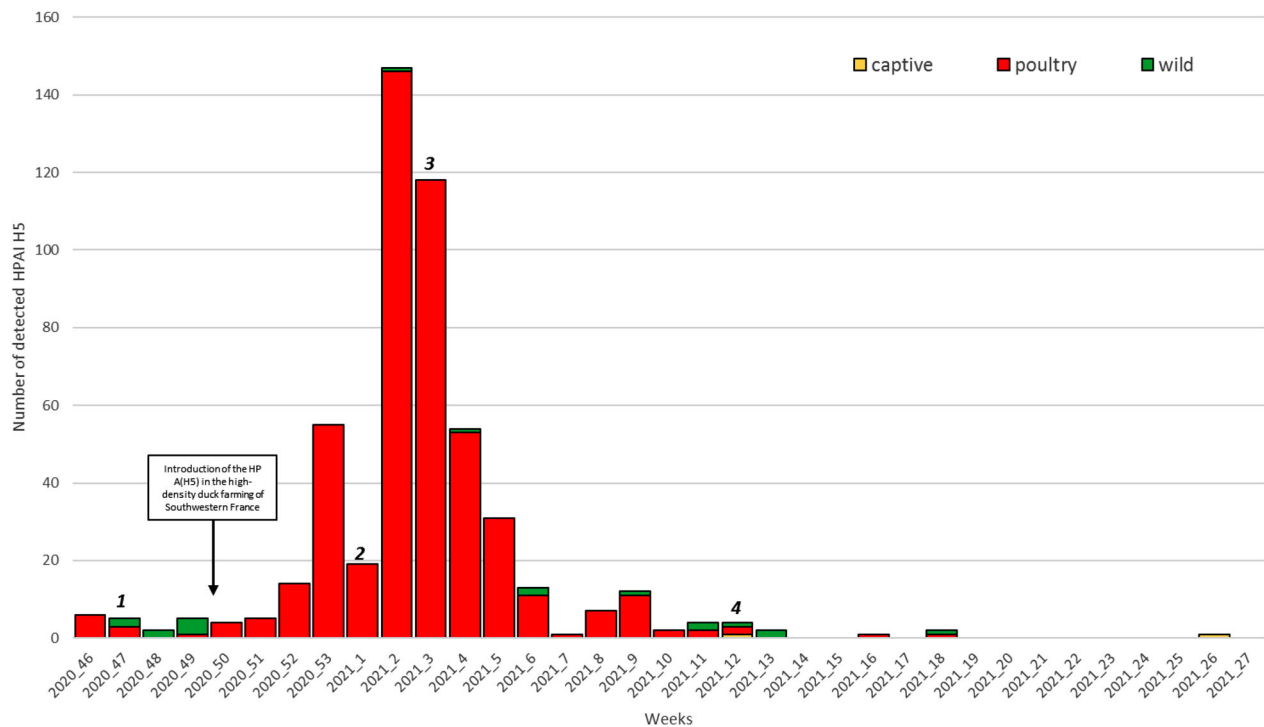


FIGURE 1 Temporal distribution of the number of detected HPAI H5 virus infection cases by week and bird categories in France. 1: High risk level for introduction of HP viruses in France. 2: Preventive culling around outbreak <3 km (DGAL/MUS/2021-4 Appui scientifique et technique de l'Anses Demande n° « 2020-AST-0176 »). 3: Preventive culling around outbreak <5 km (DGAL/SDSPA/2021-37). 4: Start of poultry repopulation.

Indeed, during these two periods, approximately 500 cases of HPAI H5N8 were identified. In addition, cases were essentially detected in duck farms in Southwestern France (which is a high risk area for avian influenza transmission between poultry establishments due to the high density of its free-range ducks farms), and only occasionally in the other regions of France in wild birds and poultry. However, contrary to the 2016–17 episode, four detections of HPAI H5N8 were detected in domestic duck farms in another high-density duck farms area in districts n°79 and n°85 during the 2020–2021 epizootic period. In contrast with the evolution observed in Southwestern France, the viral introductions in this West of France (district n°79 and N°85) were not followed by HPAI H5N8 farm-to-farm transmission. This difference in spreading patterns between these two areas may be explained by the differences in rearing structures and field conditions of duck farming (Anses, 2021). The monitoring of avian influenza in France, particularly for the duck farms, remains essential: on the one hand, to reduce the economic losses for the poultry production system by preventing an avian influenza epizootic, and on the other hand, to control the spread of AI viruses and protect birds. Moreover as observed in Russia in December 2020, with a viral detection in human host, the HPAI H5N8 virus may have a zoonotic potential (Pyankova et al., 2021). It is also crucial for public health to control the potential presence of the zoonotic makers depending on specific mutations and/or exposure conditions of humans to infected poultry. The evaluation of zoonotic potential of French H5N8, based on amino acid markers, was carried out in collaboration by Anses French AI NRL, French Public Health Institute and French National Reference Centers for viral

respiratory diseases, show a lack of relevant specific zoonotic markers (French ministry, 2021). In summary, during the entire epizootic period 2020–2021 in France, 11 viral independent introductions of HPAI H5 2.3.4.4b were identified, nine of H5N8 AI viruses in poultry, wild and captive birds, and two of H5N1 and H5N3 viruses in wild birds.

AUTHOR CONTRIBUTIONS

FXB: conceptualization, methodology, writing – original draft. EN, AS, CM, MC, PM, AvdW, AS, SLBL, SR, YL: review & editing. RB, CG, IP, KL, FS, CA, HQ: investigations. PL: data curation. YB: review & Editing and data curation. NE, BG: writing – review & editing, supervision.

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CONFLICT OF INTEREST

The authors confirm no conflict of interest.

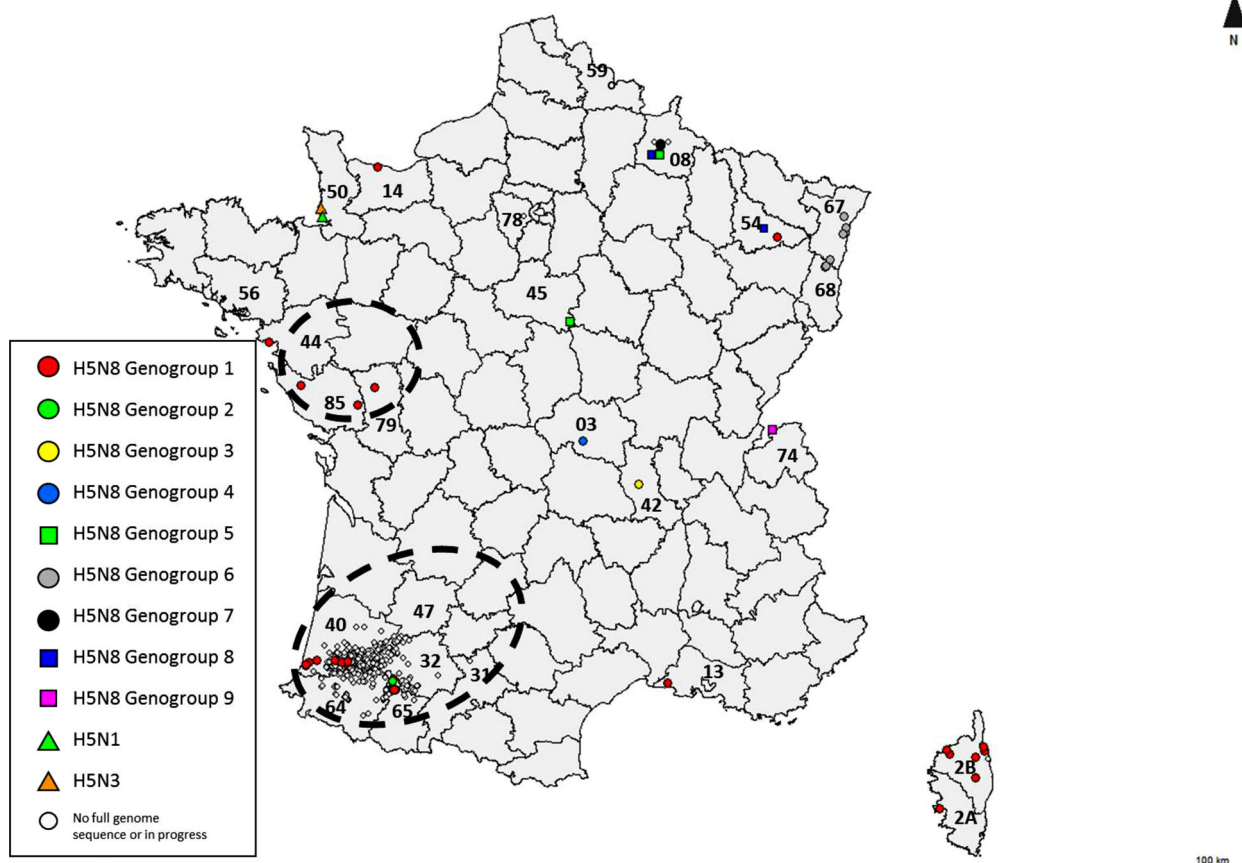


FIGURE 2 Geographical distribution of the phylogenetic groups of highly pathogenic avian influenza H5N8, H5N1 and H5N3 viruses based on the concatenated genomic segments. Each phylogenetic group or subtype is indicated by a different coloured symbol. Coloured circles and squares represent HPAI H5N8 genogroup. Green and orange triangles represent HPAI H5N1 and H5N3 viruses, respectively. Dashed circles indicate zones of high duck farm density (Agence nationale de sécurité sanitaire, 2017). French district numbers are indicated where HPAI H5 viruses have been detected.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Genbank at <https://www.ncbi.nlm.nih.gov/nucleotide/>, reference numbers OK022285 to OK022635.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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