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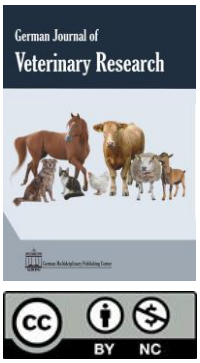
Case Report

Highly pathogenic avian influenza A (H5N1) clade 2.3.4.4b virus infection in captive bears (*Ursus thibetanus*) and in captive and wild birds, France, 2022

François-Xavier Briand[1*], Marielle Beltrame[2], Carole Guillemoto[1], Rachel Busson[1], Laetitia Pigeyre[3], Véronique Beven[1], Arnault Felten[1], Angelina Orosco[1], Patrick Daniel[4], Loic Palumbo[5], Antoine Joris[2], Yannick Blanchard[1], Audrey Schmitz[1], Eric Niqueux[1], Béatrice Grasland[1#], Yannick Simonin[6#]

[1] Anses, Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France
[2] Sigean African Reserve, Sigean, France
[3] Ingénierie et Analyse en Génétique Environnementale, Montpellier, France
[4] Laboratoire Départemental d'Analyse & de Recherche (LDAR24), Coulounieix-Chamiers, France
[5] French Biodiversity Agency (OFB), Orléans, France
[6] Pathogenesis and Control of Chronic Infections, University of Montpellier, INSERM, Etablissement Français du Sang, Montpellier, France



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***Corresponding author:**
François-Xavier Briand
francois-xavier.briand@anses.fr

# These senior authors contributed equally to this article.

Abstract

Since 2016, Europe has faced recurrent epidemics of highly pathogenic avian influenza (HPAI) A (H5N1) virus of clade 2.3.4.4b, with large numbers of deaths in wild and domestic birds. The 2022-2023 epidemic was characterized by an increase in the number of avian cases but also by an increased number of mammalian cases. Infected wild mammals were mainly marine mammals or scavengers such as foxes. Additionally, cases involving domestic mammals, like cats and farmed minks, have been documented. In December 2022, a HPAI subtype H5N1 virus belonging to clade 2.3.4.4b was identified in a captive bear and simultaneously in captive and wild birds in a wildlife park in France. Sequencing and phylogenetic analyses revealed that the bear and captive birds in the wildlife park most likely had the same origin and came directly from the infected wild birds. It is advised to monitor symptomatic domestic or captive carnivores when they come into contact with infected birds to stop the spread of the virus across species and to identify the potential mammalian-specific viral molecular markers.

Keywords: Highly pathogenic avian influenza virus, H5N1, Bears, Wild Birds, Transmission, France

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Introduction

Europe has been facing widespread outbreaks of highly pathogenic avian influenza (HPAI) virus clade 2.3.4.4b every winter for the last four years. In Europe, the epidemic period 2022-2023 was largely dominated by the H5N1 subtype, with 4300 detections in wild birds and 1080 detections in poultry (EFSA et al., 2023a). This virus caused high mortality in wild birds, such as waterfowl and seabirds, as well as in domestic poultry, resulting in significant economic losses. France was also particularly affected by the HPAI H5N1 virus, with 542 detections in wild birds and 396 detections in poultry (ESA, 2023). Among all these viruses, a wide range of genetic variability has been observed due to its ability to reassort with other influenza viruses, leading to the detection of numerous genotypes. During the epizootic period 2022-2023, at least 14 genotypes have been described in Europe, including nine in France. Particularly since 2022, increased mortality associated with the presence of this virus has been observed in various mammalian species in Europe and the Americas (Plaza et al., 2024). This increase is most likely due to the fact that the virus is more

frequent in wild birds than it was in previous years, particularly in seabirds such as Larids and Sulids, combined with increased surveillance in mammals of the circulation of the HPAI subtype H5N1 virus (EFSA et al. 2023b). Infected Mammals were mainly wild marine carnivores such as phocids, delphinids, otariids, or wild terrestrial carnivores such as felids, canids, or ursids (EFSA et al., 2023b). This study describes the detection of an H5N1 HP virus in a captive bear in a zoo in France at the end of 2022. Phylogenetic analysis of the viral genomic sequences identified in the bear and in captive and wild birds in the wildlife park at the same time enabled to determine the probable origin of the bear's contamination.

The case study

Post-mortem findings and blood sampling

On October 31, 2022, a 12-year-old Tibetan black bear (*Ursus thibetanus*) was found in Sigean Wildlife-park (southern France) in a lateral decubitus position associated with polypnea and hyperthermia (39.9°C). This bear (bear1) was anesthetized for examination, and blood samples were taken. The bear was found dead the next day, and the necropsy revealed extremely congested lungs. On November 07, a second 6-year-old Tibetan black bear (bear2) was found exhausted with respiratory distress. The animal showed signs of fatigue, anorexia, and coughing for about two weeks before fully recovering from the infection. A few days later, two other bears from the same enclosure as the two previous bears presented moderate polypnea associated with anorexia, also suggesting a similar infection. Almost concomitantly with the clinical signs observed in bears, some mortalities in wild and captive birds were observed in the wildlife park. The first avian deaths were observed on November 04, 2022, in captive pink-backed pelicans, and on November 15, 2022, others in captive pink-backed pelicans wild western jackdaws and black-headed gulls. All birds were found near the bear enclosure in the wildlife park. For bear1 and bear2, viral RNA was extracted from tracheal and sinonasal swabs using the NucleoSpin VET kit (Macherey-Nagel, Düren, Germany) and tested for HPAI (H5N1, clade 2.3.4.4b) for SARS-CoV-2 and adenoviruses (Nyaruaba et al. 2022) by digital PCR (dPCR) using the QIAcuity One-Step Viral RT-PCR kit (Qiagen, Hilden, Germany). In birds, viral RNA was extracted from cloacal and tracheal swabs using the Adiamag kit (BioX Diagnostics, Rochefort, Belgique) and tested for the M and H5 genes by rRT-PCR using the Adiavet AIV M and H5-H7. Subsequently, the RNA samples from bears and birds were analyzed by real-time RT-PCR specific for the H5 HA gene of clade 2.3.4.4b and neuraminidase (N1) genes (Hoffmann et al., 2016; Naguib et al., 2017) using Quantitect virus kit (Qiagen, Hilden, Germany).

Viral sequencing and phylogenetic analysis

One sample from each animal was found positive for H5 HA of clade 2.3.4.4b, and the full genome sequencing of detected HPAI H5N1 was performed after amplification of the eight viral genomic segments by conventional RT-PCR (Zhou et al., 2009). These viral sequences were aligned using the MAFFT software with the closest sequences of HPAI H5N1 viruses circulating in France and Europe during the winter of 2022-2023, and the sequences were subjected to phylogenetic analysis. The maximum likelihood method based on the Kimura 2-parameter model was performed to obtain phylogenetic trees with 1000 bootstrap replicates using the MEGA7 software (Kumar et al. 2008).

Histopathological and biochemical findings

Samples obtained from bear1 showed a dark red or necrotic tracheal mucosa. There was disseminated intravascular coagulation, probably of pulmonary origin, sepsis with generalized congestion. Histopathological analysis showed severe multifocal necro suppurative hepatitis, multifocal fibro-necrotic splenitis, marked pulmonary congestion, and edema. Biochemical analyses revealed severe neutropenia and thrombocytopenia, a slight increase in uremia, severe hypercreatininemia, and an increase in alanine aminotransferase. However, the biochemical results of bear2 showed a very slight neutrophilia without leukocytosis.

Pathogen detection

Virus testing of the bears was positive for HPAI (H5N1, clade 2.3.4.4b) by digital PCR (dPCR) and negative for SARS-CoV-2 and adenoviruses. Results from bear2 suggested a positivity for H5N1, clade 2.3.4.4b, but with a much lower viral load. With the reference methods for AIV

detection, results showed the presence of the HPAI H5N1 clade 2.3.4.4b virus in samples from bear1 and birds but not from the bear2 sample (Table 1), and others from Europe (Table 2).

Table 1: Detection of AIV from bears and captive/wild birds in Sigean Wildlife Park.

Sample	Case	Collection date	Species	Alternative methods for AIV detection dPCR for AIV	rRT-PCR for AIV detection (Ct value)				Complete viral sequence available
					M gene	H5 gene	H5 HP clade 2.3.4.4b	N1 gene	
23P002848	D-23-01516	31.10.2022	Bear1	positive	/	/	26.4	27.2	Yes
23P002847	D-23-01516	07.11.2022	Bear2	positive	/	/	-	-	No
22P023538	D-22-10346	15.11.2022	Pink-backed Pelican	/	18.2	15.3	17.8	17.6	Yes
22P023531	D-22-10345	15.11.2022	Black-headed Gull	/	24	21	26	25.2	Yes
22P023524	D-22-10341	15.11.2022	Western Jackdaw	/	25.6	23.1	29.3	28	No
22P023145	D-22-10048	04.11.2022	Pink-backed Pelican	/	20.3	17.1	21.6	20	Yes

Ct=Cycle threshold; /=Not Done; -= Not detected

Viral sequence analyses

Four complete viral genomes were obtained: one from the bear1, one from wild black-headed gulls, and two from captive pink-backed pelicans. Sequences are available in the GISAID database (https://www.gisaid.org) under accession nos. EPI_ISL_17233426 (bear), EPI_ISL_18722087, and EPI_ISL_18722088 (pelican) and black-headed gull (EPI_ISL_18722086). Phylogenetic analyses of HPAI H5N1 of the eight segments revealed that all viruses belonged to the A/duck/Saratov/29–02/2021–like genotype, which was the predominant virus genotype circulating in France and Europe during this epizootic period (EFSA et al. 2023b). In addition, the viral sequence from bear1 differed by 12 to 14 nucleotides (out of 13005 total nucleotides) from the sequences identified in birds from the Sigean Wildlife Park. The phylogenetic analysis of the HA segment showed that the sequences from the Wildlife-park (bear and birds) were closely related among themselves and were closely related to viruses detected from September 2022 to November 2022 in the central French departments in captive and wild birds (Figure 1).

The phylogenetic trees based on the seven remaining avian influenza segments showed the same topology. These results suggest likely contamination of the bear and captive birds of the wildlife park directly from the infected wild birds. Among the distinctive amino acid mutations observed between wild/captive birds and bear1, the E627K mutation on the PB2 protein was only identified in the H5N1 virus isolated from the bear. This mutation has previously been described as a major marker of influenza virus adaptation to mammalian hosts (Gabriel et al., 2013). The absence of this mutation in the closest viral sequences found in wild and captive birds from the Sigean Wildlife-park suggests a rapid selection of viruses with this mutation after crossing the bird/mammal species barrier as already observed (Bordes et al. 2023, Briand et al. 2023).

Conclusion

This study seems to indicate that wild birds infected with highly pathogenic avian influenza H5 can spread the infection to captive birds and bears. The diverse range of captive species in zoos, which are routinely under veterinary surveillance, may play a sentinel role in detecting the circulation of avian influenza in wild birds and its spread to other species. The early detection of highly pathogenic avian influenza in birds in and around this type of park makes it possible to limit the spread of these viruses and limit the possibility of them crossing the species barrier, thus limiting the selection of viruses with a mutation in the PB2 protein. Nevertheless, this event can occur as described here due to the diversity of birds and mammals nearby in zoos, highlighting the need for the animal keepers and veterinarians in zoos to wear individual protective equipment when handling zoo animals possibly infected by or suspected of HPAI during epizootics, to limit opportunities for inter-species transmission.

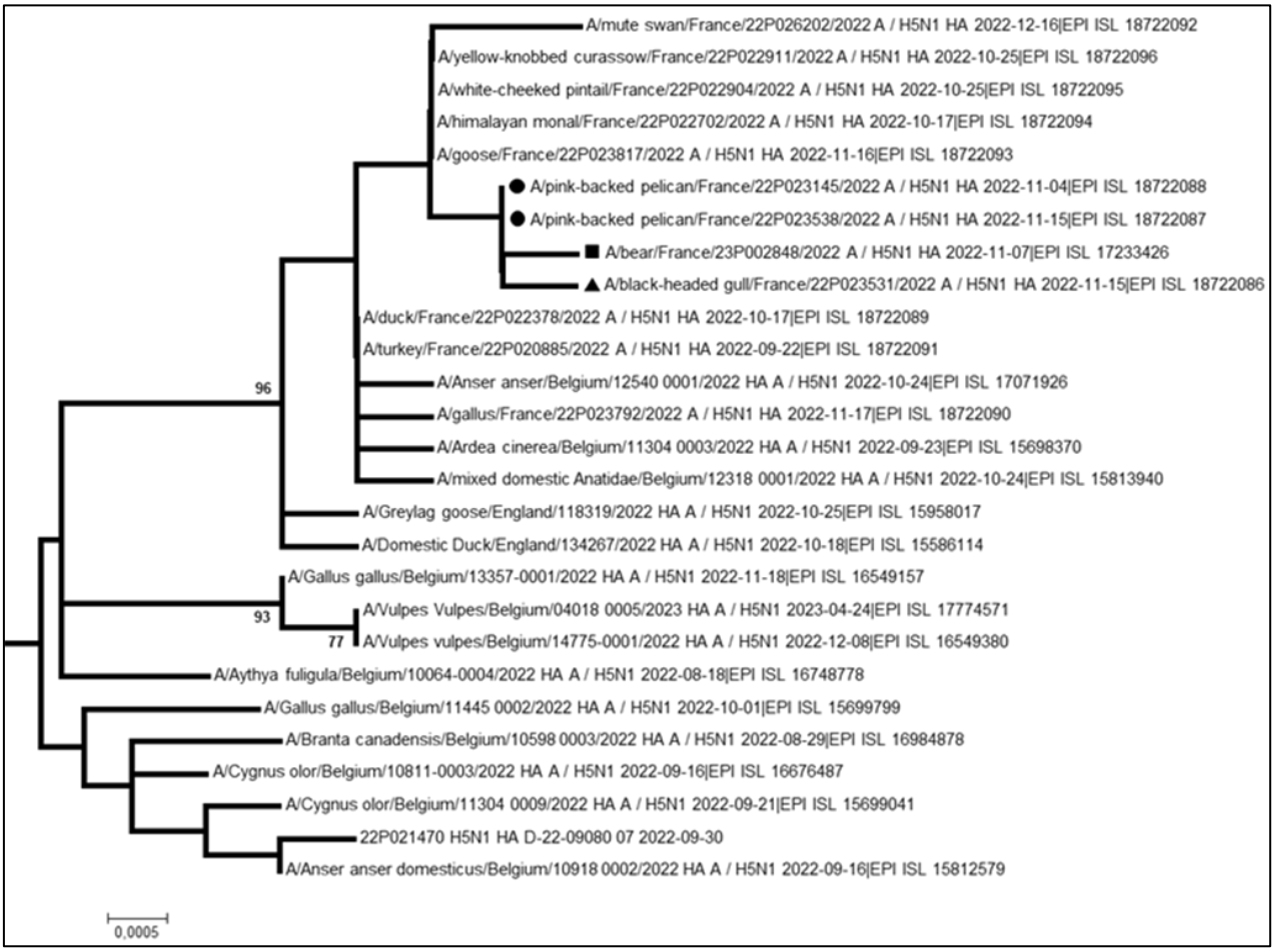

Figure 1: Phylogenetic analysis of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus detected in captive Asian black bear, France, 2022. The tree was created by using MEGA 7 software (https://megasoftware.net), and the tree was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter mode with 1,000 bootstrap replicates for HA HPAI H5N1 virus segments. All sequences belong to the A/duck/Saratov/29–02V/2021–like genotype. The black square indicates the virus sequence from the bear; the black triangle indicates the sequence from wild birds, and captive birds in the same wildlife park are indicated by the black circle. Sequences are available in the GISAID database (https://www.gisaid.org) under accession nos. EPI_ISL_17233426 (bear), EPI_ISL_18722087, and EPI_ISL_18722088 (pelican) and black-headed gull (EPI_ISL_18722086). The scale bar indicates nucleotide substitutions per site. Marked sequences are identified in the Sigean Wildlife Park.

Article Information

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Conflict of Interest. The authors declare no conflict of interest.

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Table 2. Additional information from the GISAID database for the highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus detected in a captive bear in Europe, 2022.

Isolate ID	Isolate Name	Subtype	Country	Host	Collection date	Submitting lab date
EPI_ISL_15586114	A/Domestic_Duck/England/134267/2022	H5N1	England	Duck	2022-10-18	2022-10-31
EPI_ISL_15958017	A/Greylag_goose/England/118319/2022	H5N1	England	Goose	2022-10-25	2022-11-30
EPI_ISL_16748778	A/Aythya_fuligula/Belgium/10064-0004/2022	H5N1	Belgium	Aythya fuligula	2022-08-18	2023-02-01
EPI_ISL_15698370	A/Ardea_cinerea/Belgium/11304_0003/2022	H5N1	Belgium	Wild bird	2022-09-23	2022-11-08
EPI_ISL_15812579	A/Anser_anser_domesticus/Belgium/10918_0002/2022	H5N1	Belgium	Anser anser domesticus	2022-09-16	2022-11-18
EPI_ISL_16549157	A/Gallus_gallus/Belgium/13357-0001/2022	H5N1	Belgium	Gallus gallus domesticus	2022-11-18	2023-01-18
EPI_ISL_16549380	A/Vulpes_vulpes/Belgium/14775-0001/2022	H5N1	Belgium	Canine	2022-12-08	2023-01-18
EPI_ISL_16676487	A/Cygnus_olor/Belgium/10811-0003/2022	H5N1	Belgium	Cygnus olor	2022-09-16	2023-01-26
EPI_ISL_16984878	A/Branta_canadensis/Belgium/10598_0003/2022	H5N1	Belgium	Branta canadensis	2022-08-29	2023-02-21
EPI_ISL_17774571	A/Vulpes_Vulpes/Belgium/04018_0005/2023	H5N1	Belgium	Canine	2023-04-24	2023-06-07
EPI_ISL_15699041	A/Cygnus_olor/Belgium/11304_0009/2022	H5N1	Belgium	Cygnus olor	2022-09-21	2022-11-08
EPI_ISL_15699799	A/Gallus_gallus/Belgium/11445_0002/2022	H5N1	Belgium	Gallus gallus domesticus	2022-10-01	2022-11-08
EPI_ISL_15813940	A/mixed_domestic_Anatidae/Belgium/12318_0001/2022	H5N1	Belgium	Other avian	2022-10-24	2022-11-18
EPI_ISL_17071926	A/Anser_anser/Belgium/12540_0001/2022	H5N1	Belgium	Greylag goose	2022-10-24	2023-03-02