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Source: Journal of Wildlife Diseases, 61(3) : 792-796

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/JWD-D-24-00176

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Journal of Wildlife Diseases, 61(3), 2025, pp. 792–796

Highly Pathogenic Avian Influenza in Northern Fulmars (*Fulmarus glacialis*) in the Netherlands

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ABSTRACT: We report highly pathogenic avian influenza H5 virus infection in 10 Northern Fulmars (*Fulmarus glacialis*) found dead throughout January and February 2024 in the Netherlands. Five birds were infected with the H5N5 subtype, notable for markers of adaptation to mammals. Continuous infectious disease surveillance remains important in wild birds.

Highly pathogenic avian influenza (HPAI) is a threat to wild bird health, and outbreaks in seabird breeding colonies have caused numerous mass mortality episodes (Caliendo et al. 2024; Fusaro et al. 2024). The breeding season is a particularly vulnerable time for colonial birds because of increased proximity between the animals and the presence of immunologically naïve chicks. However, HPAI virus infection in seabirds can also occur outside the breeding season, with infected seabirds dying unseen, at sea, and over different days and locations. As a result, collection of carcasses for testing is more difficult and diagnosis of infection is more challenging, as in this report. We report HPAI H5 virus infection in 10 Northern Fulmars (*Fulmarus glacialis;* hereafter Fulmar) found dead in January and February 2024 at various locations in the Netherlands and highlight the importance of continued HPAI surveillance in seabirds outside the breeding season.

Fulmars have been used for ecologic monitoring for marine litter, and their carcasses are regularly collected and investigated for this purpose (Kühn et al. 2024). In 2021–24, Wageningen Marine Research Institute collected 33 dead Fulmars investigated for plastic ingestion monitoring (2021: $n=4$, 2023: $n=8$, 2024: $n=21$). All birds were found dead in various states of decomposition on Dutch coasts, without any signs of unusual mortality. Because of the seemingly unrelated pattern of mortalities, HPAI virus infection was not initially suspected.

During the dissection of the birds, we collected a combined tracheal–cloacal swab and a brain swab (in 2024, only for the first 10 birds were brain swabs obtained) and tested all swabs by real-time PCR (Poen et al. 2019; Chestakova et al. 2023). For the brain swab we swabbed all brain parts from a midsagittal section. We tested the swabs in an in-house-developed triplex reaction for the influenza A virus matrix gene combined with the H5 gene, plus phocine distemper virus as an internal control (Chestakova et al. 2023).

For each of the same 10 birds we also collected brain, heart, and liver samples for histopathology and immunohistochemistry. Tissues were fixed in 10% neutral buffered formalin, H&E stained, and stained for influenza A nucleoprotein expression (Vreman et al. 2022). We detected HPAI H5N1 in two birds and HPAI H5N5 virus in five birds by direct sequencing; the remaining three birds were HPAI H5 virus positive but we were unable to determine the subtype (Table 1). Pathologic lesions in infected birds were multifocal encephalitis (7/10) with foci of gliosis, neuronal degeneration, and necrosis; viral antigen was present in the nucleus and cytoplasm of several neurons (Fig. 1). The heart in 5/10 birds showed multifocal to focally extensive myocardial necrosis; several myocardial cells presented viral antigen.

Fulmars are opportunistic and generalist surface feeders. Their diet ranges from fish, squid, and zooplankton to scavenged discards and offal from fishing vessels or scavenged carcasses floating on the sea surface (Mallory et al. 2012). Therefore, possible routes for HPAI virus infection may include scavenging infected carcasses drifting offshore or contact with other migratory birds. A source of infection was not identified in

TABLE 1. Bird number, date and location of collection, and results from real-time PCR (q-PCR) of the influenza A H5-HA gene-fragment in combined tracheal–cloacal (Cl-Tr) swabs and brain swabs of highly pathogenic avian influenza H5 virus-infected wild Northern Fulmars (*Fulmarus glacialis*) found dead in the Netherlands in 2021–24. The cutoff for cycle threshold (Ct) values was 40. Virus pathotype and sequences are publicly accessible via the Global Initiative on Sharing of Influenza Data (GISAID 2024).

Bird no.	Collection date	Collection location	Cl-Tr swab[a]	Brain swab[a]	Virus pathotype	Sequence (GISAID accession no.)
NET-2021-092	6 April 2021	Noordburen; Hippolythushoef	ND[b]	ND	NT[c]	NT
NET-2021-089	16 April 2021	Zierikzee; Zeeland	ND	ND	NT	NT
NET-2021-090	30 October 2021	Ameland	ND	ND	NT	NT
NET-2021-091	9 December 2021	Kreupel; Ijsselmeer	ND	ND	NT	NT
NET-2023-026	28 May 2023	Ameland	ND	ND	NT	NT
NET-2023-027	28 May 2023	Ameland	ND	ND	NT	NT
NET-2023-028	28 May 2023	Ameland	ND	ND	NT	NT
NET-2023-032	8 June 2023	Ameland	ND	ND	NT	NT
NET-2023-025	8 July 2023	Petten	ND	ND	NT	NT
NET-2023-031	8 August 2023	Texel	ND	ND	NT	NT
NET-2023-024	1 November 2023	Ameland	ND	ND	NT	NT
NET-2023-030	20 December 2023	Texel	ND	ND	NT	NT
NET-2024-005	16 January 2024	Texel, Paal 12	ND	32	HPAI H5N5	NT
NET-2024-007	20 January 2024	Texel, Paal 8	23	16	HPAI H5N1	NT
NET-2024-002	23 January 2024	Texel, Paal 13	31	31	HPAI H5Nx	NT
NET-2024-004	24 January 2024	Texel, Paal 15	19	20	HPAI H5N5	A/Northern Fulmar/Netherlands/1/2024 (EPI_ISL_19053252)
NET-2024-006	24 January 2024	Texel, Paal 21	31	32	HPAI H5Nx	NT
NET-2024-001	25 January 2024	Texel, Ferry Harbour	19	19	HPAI H5N5	NT
NET-2024-003	25 January 2024	Texel, Ferry Harbour	21	20	HPAI H5N5	A/Northern Fulmar/Netherlands/2/2024 (EPI_ISL_19053253)
NET-2024-010	27 January 2024	Texel, Paal 21	33	NT	HPAI H5Nx	NT
NET-2024-009	28 January 2024	Texel, Paal 6	32	36	HPAI H5N5	A/Northern Fulmar/Netherlands/3/2024 (EPI_ISL_19053254)
NET-2024-008	7 February 2024	Zierikzee; Zeeland	24	16	HPAI H5N1	A/Northern Fulmar/Netherlands/4/2024 (EPI_ISL_19053265)
NET-2024-014	26 March 2024	Den Hoorn Texel	ND	NT	NT	NT

TABLE 1. Continued.

Bird no.	Collection date	Collection location	Cl-Tr swab[a]	Brain swab[a]	Virus pathotype	Sequence (GISAID accession no.)
NET-2024-017	30 March 2024	Texel Paal 19	ND	NT	NT	NT
NET-2024-023	19 April 2024	Ouddorp	ND	NT	NT	NT
NET-2024-024	13 May 2024	Ameland	ND	NT	NT	NT
NET-2024-012	14 May 2024	Ameland	ND	NT	NT	NT
NET-2024-013	5 June 2024	Bloemendaal	ND	NT	NT	NT
NET-2024-020	12 June 2024	Texel	ND	NT	NT	NT
NET-2024-011	10 September 2024	TX De Hors	ND	NT	NT	NT
NET-2024-019	11 September 2024	TX P22	ND	NT	NT	NT
NET-2024-016	14 September 2024	Kwade Hoek	ND	NT	NT	NT
NET-2024-015	17 September 2024	Schiermonnikoog	ND	NT	NT	NT

[a] qPCR H5 Ct value.
[b] ND = not detected.
[c] NT = not tested.

our study. The H5N1 virus was genotype EA-2021-AB. The HPAI H5N1 virus genome sequence that we obtained from one Fulmar was 99–100% identical for the various gene segments to the genome sequences of viruses from a Mute Swan (*Cygnus olor*), a Barnacle Goose (*Branta leucopsis*), and a Eurasian Oystercatcher (*Haematopus ostralegus*) in England, Sweden, and Belgium, respectively (Fusaro et al. 2024). The H5N5 virus was genotype EA-2021-I. The HPAI H5N5 virus sequences that we obtained from three Fulmars were closely related to viruses from a Eurasian Sparrowhawk (*Accipiter nisus*) and a Common Buzzard (*Buteo buteo*) in England and from an unspecified gull in Germany (Fusaro et al. 2024). Notably, H5N5 viruses have a 22-amino-acid deletion in the stalk domain of the neuraminidase protein and a E627K substitution in the viral protein PB2 (Fusaro et al. 2024). These genetic changes are uncommon in viruses of wild birds; they are generally considered to be markers of host adaptation to mammals and poultry. Related viruses have recently been detected in mammals in Canada and in wild birds over a wide geographic range including Canada, Greenland, Iceland, and northwest Europe (Erdelyan et al. 2024). No avian influenza outbreaks occurred in poultry in the Netherlands around the collection time frame (16 January to 7 February 2024) of the HPAI H5Nx-positive Fulmars. However, HPAI H5N5 virus was retrospectively detected in a Pine Marten (*Martes martes*) in Castricum (a seaside location in Nord-Holland, the Netherlands) on 1 February 2024 (Shu and McCauley 2017; virus isolate name: A/Pine Marten/Netherlands/1/2024, virus isolate ID: EPI_ISL_19361216, GISAID 2024). The animal showed neurologic signs before death. It is possible that the marten was infected after contact with an infected bird.

The European Fulmar population consists of about 3,380,000–3,500,000 breeding pairs, and its population status is classified as Least Concern by the International Union for Conservation of Nature Red List (BirdLife International 2018). Nevertheless, recent declines have been observed, and a further population decline up to 42% is expected by 2060 (BirdLife International 2018). A similar trend has

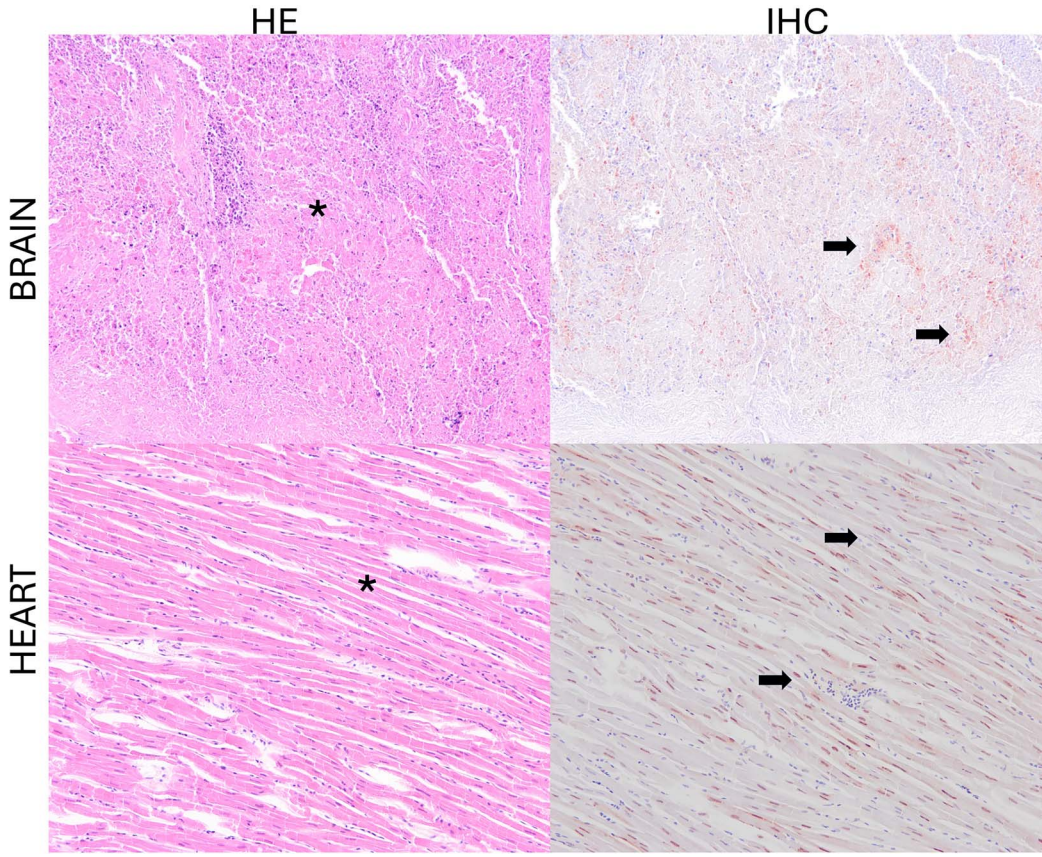

FIGURE 1. Histopathologic changes and influenza virus antigen expression in tissues of highly pathogenic avian influenza H5N5 virus-infected Northern Fulmar (*Fulmarus glacialis*) found dead in the Netherlands in 2024. Tissue sections in the left column were stained with H&E (labeled HE in the image). Serial tissue sections in the right column were stained to detect influenza virus antigen by immunohistochemistry (IHC). In all tissues we found necrosis (asterisks) and inflammation associated with virus antigen expression (arrows).

been observed for the Netherlands, with about 6,100–21,900 wintering individuals. Over the past 2 decades, the number of Fulmars in the Dutch North Sea has decreased significantly (Sovon 2024). Anthropogenic activities have been suggested as the major cause for its decline in Europe. Highly pathogenic avian influenza may represent another contributing factor in this process, although since 2022 HPAI-positive Fulmars have only sporadically been reported to the World Organisation for Animal Health (Serafini et al. 2023).

Our findings highlight the importance of continuous infectious disease surveillance, including surveillance for HPAI in wild birds, and particularly in seabirds. Whenever increased mortalities are observed, birds should be screened for HPAI—not only during mass mortality events, but also when mortalities are more dispersed. We propose that continuity in infectious disease surveillance in wildlife can be achieved by working in combination with other, differently focused, monitoring projects such as beached bird surveys, or in our case, plastic pollution monitoring.

We thank the members of the AI-Impact group for their contribution. We thank our surveyors for the collection of beached Fulmars and Jan van Franeker, André Meijboom, and Oliver Bittner for their help during dissections. This research was financed by the European Union's Horizon 2020 research and innovation programme grant no. 101084171. Fulmar plastic monitoring in the Netherlands

is supported financially by Rijkswaterstaat of the Netherlands Ministry of Infrastructure and Water Management, and avian influenza monitoring is supported through the Strengthening One Health surveillance (Strong1H) EU4Health Project 101132302—[EU4H-2022-DGA-MS-IBA3]. All data are available in the article and GISAID (2024).

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Submitted for publication 4 October 2024.
Accepted 27 January 2025.