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Short communication

Highly pathogenic avian influenza H5N1 virus outbreak among common terns (Sterna hirundo) in Namibia, 2025-2026

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Abstract

Highly pathogenic avian influenza A(H5N1) viruses of clade 2.3.4.4b continue to spread globally, causing major outbreaks in wild birds and poultry. In Africa, however, genomic data remain limited, restricting understanding of viral introduction routes and circulation patterns. Here, we report the whole-genome characterisation of an HPAI A(H5N1) virus detected in a common tern (*Sterna hirundo*) found dead on the Namibian coast during the most recent avian influenza outbreak recorded in the country. Viral RNA was subjected to whole-genome sequencing using the Illumina Viral Surveillance Panel v2 on a NextSeq 1000 platform. Complete or near-complete sequences were obtained for all eight genome segments and deposited in GenBank. Phylogenetic analyses, performed using African clade 2.3.4.4b H5Nx sequences and the closest related sequences identified through database searches, showed that the Namibian virus belonged to clade 2.3.4.4b and clustered within the EA-2024-DI.2 subgenotype. Across all segments, the virus grouped with contemporary European EA-2024-DI.2 viruses circulating during the 2024–2025 epidemic wave, supporting a likely Eurasian origin. For six of the eight segments, it also clustered closely with an EA-2024-DI.2 virus detected in a gull-billed tern in Uganda in December 2024. Molecular analysis identified a polybasic haemagglutinin cleavage site consistent with high pathogenicity and a mutational profile broadly similar to contemporary EA-2024-DI.2 viruses. The HA substitution, associated in previous studies with increased binding to mammalian-type α2-6 receptors, may warrant further investigation. These findings highlight the role of migratory seabirds in H5N1 dissemination and reinforce the need for strengthened genomic surveillance in African wild birds and poultry.

Keywords

Avian influenza, Namibia, Common terns, H5N1, Clade 2.3.4.4b

Highly pathogenic avian influenza (HPAI) viruses of the H5 subtype continue to represent a major threat to both the global poultry industry and wild bird populations, causing substantial economic losses and severe ecological consequences worldwide. In recent years, clade 2.3.4.4b H5Nx viruses of the Goose/Guangdong/1/96 (Gs/GD) lineage have been responsible for extensive outbreaks in domestic poultry, as well as for unprecedented mortality events in free-ranging wild birds across Asia, Europe, Africa, and the Americas, highlighting their remarkable capacity for intercontinental spread and adaptation to multiple avian hosts (Koopmans et al., 2024). H5N1 viruses belonging to the Gs/GD lineage were first identified in domestic geese in China in 1996. Following their emergence, the Gs/GD H5N1 lineage continued to circulate, evolve, and spread among poultry populations across Asia. The 2005 Gs/GD H5N1 outbreak at Qinghai Lake in migratory waterbirds marked an epidemiological turning point in terms of the involvement of migratory wild birds, with subsequent spread along major flyways contributing to the dissemination of the virus beyond Asia. Since then, multiple transcontinental epidemic waves have occurred, and the virus has further evolved into multiple clades and genotypes (Sonnberg et al., 2013; Xie et al., 2023). The spread of H5Nx HPAI,

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particularly clade 2.3.4.4b viruses, has had a profound impact on wildlife, affecting an unprecedented diversity of avian species (Kuiken & Cromie, 2022). Since the first introduction of this clade into Africa in December 2020, outbreaks caused by clade 2.3.4.4b H5Nx HPAI viruses have been reported in several African countries, affecting both domestic poultry and wild bird populations. The initial incursions, linked to European strains, involved A/H5N8 genotype EA-2020-A and A/H5N1 genotype EA-2020-C (Fusaro et al., 2024). From 2023 to 2025, clade 2.3.4.4b H5N1 HPAI outbreaks were reported in domestic poultry and backyard flocks across several sub-Saharan African countries, including Senegal, The Gambia, Guinea, Niger, Nigeria, Togo, Burkina Faso, Gabon, Liberia, Ghana, South Africa, and Botswana, while concurrent infections were also documented in wild coastal seabirds throughout the region (Lo et al., 2022; Abolnik, 2025; Fasina et al., 2025). Concurrently, multiple wild bird species in South Africa, including African penguins, gulls, pelicans, cormorants, ibises, and raptors, tested positive for clade 2.3.4.4b H5N1 HPAI virus. In Namibia, evidence of HPAI circulation in wild birds was reported in January 2019, when a colony of African penguins was found to be infected with clade 2.3.4.4b H5N8 virus (Molini et al., 2020). Subsequently, in January 2022, a new outbreak caused by clade 2.3.4.4b H5N1 virus was detected in Cape cormorants (Molini et al., 2023). The present report describes an H5N1 HPAI event involving a common tern in Namibia and presents the results of the genome characterisation of the detected clade 2.3.4.4b virus. Between December 2025 and January 2026, approximately 40 seabirds, all identified as common terns (*Sterna hirundo*), were found dead or observed showing clinical signs compatible with HPAI on Mercury Island near Lüderitz and at Dolphin Beach near Walvis Bay. Reported clinical signs included depression, incoordination, respiratory distress, and inability to fly. The carcass of a common tern recovered at Dolphin Beach, near Walvis Bay in the Erongo Region, was collected, refrigerated, and submitted to the Central Veterinary Laboratory for diagnostic investigation on 30 January 2026. A pooled organ sample, including liver, lung, trachea, and intestinal tissue, was homogenised in 1 mL of sterile phosphate-buffered saline using a TissueLyser LT system (Qiagen, Hilden, Germany). Viral RNA was extracted from 200 µL of homogenate using the High Pure Viral Nucleic Acid Kit (Roche, Basel, Switzerland) and eluted in a final volume of 100 µL. Detection of influenza A virus was performed by reverse transcription quantitative PCR (RT-qPCR) targeting the matrix (M) gene using a commercial assay, the Genesig Advanced Kit Influenza A Virus (M1) (Primerdesign Ltd., Southampton, UK). Purified RNA was subsequently sent to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), Teramo, Italy, for whole-genome sequencing. Library preparation was performed using the Illumina Viral Surveillance Panel v2 (Illumina Inc., San Diego, CA, USA), and sequencing was carried out on a NextSeq 1000 platform using the NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (300 cycles) (Illumina Inc., San Diego, CA, USA). This generated 999,400 paired-end reads of 150 bp, with a mean Q-score of 36.82. Bioinformatic analyses were performed using the GenPat platform (https://genpat.izs.it/). The best-matching reference sequence for each genomic segment was identified using the CZ ID platform (https://czid.org/) and subsequently used for reference-based mapping with iVar v1.4.4 (Grubaugh et al., 2019). Complete or near-complete consensus sequences were obtained for all eight genomic segments, with horizontal coverage (Hcov) ranging from 96.43% to 100% and vertical coverage (Vcov) ranging from 82.88× to 220.58×. The sequences were deposited in GenBank under accession numbers PZ387115, PZ387138, PZ387141–PZ387144, PZ387148, and PZ387149. For phylogenetic analysis, all clade 2.3.4.4b H5Nx sequences from Africa were downloaded and added to the segment-specific datasets, alongside the ten most closely related sequences, which were selected based on BLAST searches of the Namibian virus sequences against the GISAID EpiFlu database (https://gisaid.org/).

Each dataset was aligned using MAFFT v7.525 (https://mafft.cbrc.jp/alignment/software/). Phylogenetic trees were inferred under a maximum-likelihood (ML) framework using IQ-TREE multicore v2.3.6 (Minh et al., 2020), with automatic selection of the best-fit substitution model and branch support assessed by ultrafast bootstrap analysis with 1,000 replicates (Hoang et al., 2018). The resulting trees were visualised and annotated using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Viral genotype assignment was performed using Genin2 (https://github.com/izsvenezie-virology/genin2). Amino acid substitutions and molecular markers associated with zoonotic potential, virulence, and antiviral resistance were identified using FluMut (Giussani et al., 2025). Examination of the HA amino acid sequence identified the polybasic cleavage site motif PLREKRRKRGLF, consistent with highly pathogenic avian influenza viruses. Phylogenetic analyses demonstrated that the virus belonged to clade 2.3.4.4b and clustered within the EA-2024-DI.2 subgenotype across all gene segments (Figure 1 and Supplementary Figure 1). The EA-2024-DI genotype likely emerged in Europe during late 2023 and subsequently became one of the predominant H5N1 genotypes circulating during the 2024–2025 epidemic wave in Europe (EFSA, 2025). The progressive accumulation of mutations resulted in the emergence of two descendant subgenotypes, designated EA-2024-DI.1 and EA-2024-DI.2, which circulated extensively in Europe during the 2024–2025 epidemic wave (EFSA AHAW Panel, 2025; EFSA, 2025). The Namibian virus grouped consistently with 2024–2025 European EA-2024-DI.2 viruses across all genomic segments, supporting a likely Eurasian origin for this strain. Interestingly, the Namibian virus clustered closely with an EA-2024-DI.2 strain detected in Uganda in December 2024 in a gull-billed tern (*Gelochelidon nilotica*) for six of the eight gene segments (Figure 1 and Supplementary Figure 1). The relatively long branch length separating

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the Namibian virus from the Ugandan and European viruses highlights substantial surveillance gaps across Africa. Consequently, the precise route of introduction and the extent of viral circulation before detection in Namibia remain unclear. Preliminary whole-genome sequencing and phylogenetic analyses indicate that genotype EA-2024-DI had already been detected in southern Africa, with reports from South Africa in mid-2025 (Abolnik, 2025). However, the lack of publicly available genetic sequences from that event precludes any direct comparison and prevents assessment of the genetic relatedness between those viruses and the virus identified in Namibia.

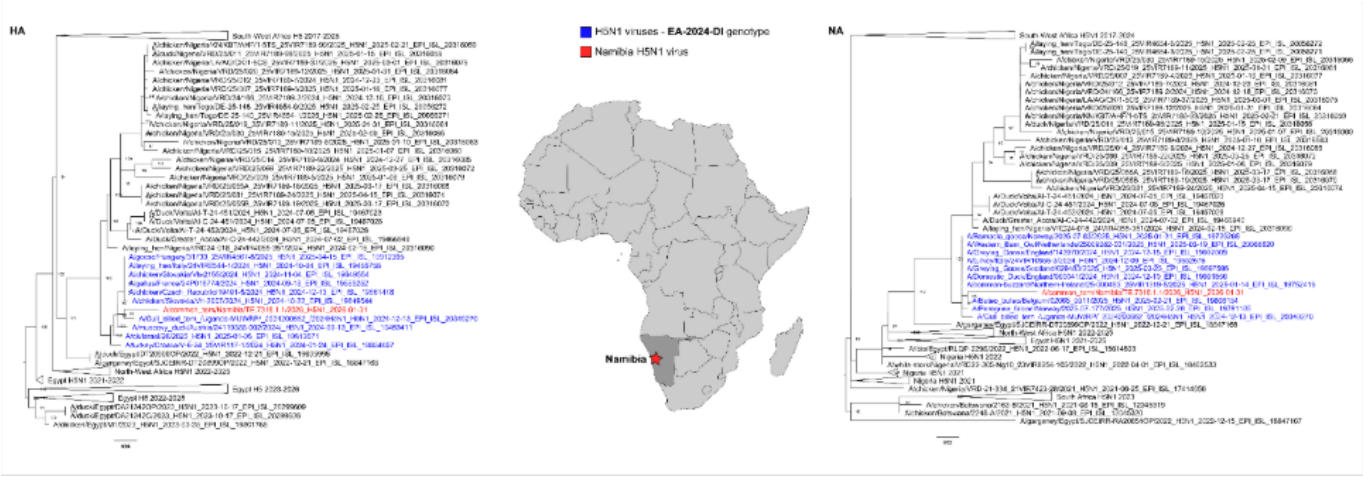

Figure 1. Maximum-likelihood phylogenetic trees based on the HA and NA gene segments of the HPAI A(H5N1) virus detected in Namibia in January 2026, highlighted in red, and related EA-2024-DI strains identified by BLAST analysis, highlighted in blue. Ultrafast bootstrap values greater than 80 are indicated next to the nodes.

While the exact pathway by which this genotype, which originally emerged in Europe, may have reached the Namibian coast remains difficult to determine, Namibia is a well-documented wintering area for common terns breeding in the Western Palearctic. Most European populations of common tern migrate south along the East Atlantic Flyway, with wintering areas extending from West Africa to southern Africa, including Namibia and South Africa. Tracking studies have also shown alternative routes, including an eastern pathway through the eastern Mediterranean and Red Sea towards East Africa, Mozambique, and South Africa (Kralj et al., 2020). This migratory connectivity supports the relevance of common terns as a species at risk of exposure to HPAI H5N1 genotypes circulating in Europe and their potential involvement in the introduction of the virus into southern Africa. Although the common tern is currently classified as Least Concern globally, the detection of HPAI H5N1 in this species in Namibia is relevant from a conservation perspective. Common terns are long-distance migratory, colony-breeding seabirds, and recent outbreaks in Europe have shown that HPAI H5N1 can cause substantial mortality in this species, including losses of breeding adults and chicks, with severe impacts on local colonies (Pohlmann et al., 2023). The Namibian detection should therefore be regarded as a warning signal, underscoring the need to maintain targeted surveillance and a high level of alert in coastal areas, especially during migration and non-breeding periods, to support early detection and the protection of coastal seabird populations. Analysis of amino acid substitutions revealed a mutational profile largely consistent with that of other contemporary European EA-2024-DI.2 viruses (Table I). Among the detected substitutions, the HA:T188I mutation identified within the haemagglutinin protein appeared to be relatively uncommon among currently available EA-2024-DI.2 sequences and may therefore deserve further investigation to clarify its possible biological significance. In the literature, this substitution has been associated with increased in vitro binding to mammalian-type α2-6 sialic acid receptors, indicating enhanced affinity for receptors commonly expressed in the human respiratory tract, although no increase in in vivo transmissibility has been demonstrated (Yang et al., 2007; Suttie et al., 2019). Overall, the present study provides additional evidence of the continued transboundary spread of H5N1 clade 2.3.4.4b viruses into Africa and underlines the importance of strengthening active surveillance programmes in both wild birds and domestic poultry across the continent. Enhanced genomic surveillance based on next-generation sequencing approaches will be essential to improve early detection of emerging variants, better understand viral evolution and dispersal pathways, and support integrated One Health preparedness strategies.

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Protein	Marker	Effect	Literature
HA*	K218Q, S223R	Increased virus binding to α2-3	Guo et al., 2017
		Increased virus binding to α2-6	Suttie et al., 2019
	S107R, T108I	Increased pH of fusion	Suttie et al., 2019
		Increased virulence in chickens	Wessels et al., 2018
		Increased virulence in mice	
	S133A	Increased pseudovirus binding to α2-6	Suttie et al., 2019
			Yang et al., 2007
	S133A, T188I	Increased pseudovirus binding to α2-6	Suttie et al., 2019
			Yang et al., 2007
	S154N	Increased virus binding to α2-6	Suttie et al., 2019
			Wang et al., 2010
	T156A	Increased transmission in guinea pigs	Gao et al., 2009
		Increased virus binding to α2-6	Suttie et al., 2019
			Wang et al., 2010
	T188I	Increased pseudovirus binding to α2-6	Suttie et al., 2019
			Yang et al., 2007
M1	I43M	Increased virulence in chickens	Nao et al., 2015
		Increased virulence in ducks	Suttie et al., 2019
		Increased virulence in mice	
	N30D	Increased virulence in mice	Fan et al., 2009
			Suttie et al., 2019
	T215A	Increased virulence in mice	Fan et al., 2009
			Suttie et al., 2019
NP	A184K	Enhanced interferon response	Suttie et al., 2019
		Increased replication in avian cells	Wasilenko et al., 2009
		Increased virulence in chickens	
NS1	C138F	Decreased interferon response	Li et al., 2018
		Increased viral replication in mammalian cells	Suttie et al., 2019
	C138F, K55E, K66E	Decreased interferon response	Li et al., 2018
		Enhanced replication in mammalian cells	Suttie et al., 2019
	I106M	Increased viral replication in mammalian cells	Ayllon et al., 2014
		Increased virulence in mice	Suttie et al., 2019
	L103F, I106M	Increased virulence in mice	Kuo et al., 2009
			Spesock et al., 2011
			Suttie et al., 2019
	N205S, NS-2:T48A	Decreased antiviral response in ferrets	Imai et al., 2010
			Suttie et al., 2019
	P42S	Decreased antiviral response in mice	Jiao et al., 2008
		Increased virulence in mice	Suttie et al., 2019
	V149A	Decreased interferon response in chickens	Li et al., 2006;
		Increased virulence in chickens	Suttie et al., 2019
PA	N383D	Increased polymerase activity in avian cells	Song et al., 2011
		Increased polymerase activity in mammalian cells	Song et al., 2015
			Suttie et al., 2019
	N409S	Increased polymerase activity in mammalian cells	Suttie et al., 2019
		Increased replication in mammalian cells	Yamayoshi et al., 2014
	S37A	Increased polymerase activity in mammalian cells	Suttie et al., 2019
			Yamayoshi et al., 2014
PB1	D3V	Increased polymerase activity in avian cells	Elgendy et al., 2017
		Increased polymerase activity in mammalian cells	Suttie et al., 2019
		Increased replication in avian cells	
		Increased replication in mammalian cells	
	D622G	Increased polymerase activity in mammalian cells	Feng et al., 2015
		Increased polymerase activity in mice	Suttie et al., 2019
		Increased virulence in mice	
PB2	I292V	Increased polymerase activity in mammalian cells	Suttie et al., 2019
			Xiao et al., 2016
		Increased virulence in mice	Gao et al., 2019
			Suttie et al., 2019
	K389R	Increased polymerase activity in mammalian cells	Hu et al., 2017
		Increased replication in mammalian cells	Suttie et al., 2019
	L89V, G309D	Increased polymerase activity in mammalian cells	Li et al., 2009
		Increased virulence in mice	Suttie et al., 2019
	L89V, G309D, T339K, R477G, I495V, K627E, A676T	Increased polymerase activity in mammalian cells	Li et al., 2009
		Increased virulence in mice	Suttie et al., 2019
	V598T	Increased polymerase activity in mammalian cells	Hu et al., 2017
		Increased replication in mammalian cells	Suttie et al., 2019
		Increased virulence in mice	

Table I. List of molecular markers with a potential impact on the biological characteristics of the HPAI A(H5N1) virus detected in Namibia, as identified using the FluMut tool. *H5 numbering

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Ethical approval

Ethical approval was not required, as the samples were obtained from a dead bird collected by Namibian State Veterinarians during the most recent avian influenza outbreak recorded in Namibia.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

Conceptualisation: UM, EH; Methodology: MA, MM; Formal analysis: MM, LC, BS, LM, IM, PC, MD; Investigation: LC, EH; Writing original draft preparation: UM, IM, MM; Writing, review and editing: UM, IM; Supervision: UM, EH

All authors have read and agreed to the published version of the manuscript.

Data availability

The data supporting the findings of this study are available within the article.

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