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Neurotropic Highly Pathogenic Avian Influenza A(H5N1) Virus in Red Foxes, Northern Germany

Appendix

Materials and Methods

RNA extraction, PCR investigations and Next Generation Sequencing (NGS) analyses

Viral RNA was extracted from the brain samples with the NucleoMag VET Kit (Macherey-Nagel) on a KingFisher 96-Flex machine, and real-time PCR targeting the viral M segment was performed with a commercial kit (Virotype Influenza A RT-PCR Kit, Indical Bioscience) according to the manufacturer´s instructions. A second real-time PCR was used to confirm the H5 subtype in the samples with the following primers and probe (AIV-H5LH1: 5‘-ACA TAT GAC TAC CCA CAR TAT TCA G-3‘, AIV-H5RH1: 5`-AGA CCA GCT AYC ATG ATT GC-3‘, 5`-FAM-TCA ACA GTG GCG AGT TCC CTA GCA-TAMRA-3‘) under these conditions: 50°C, 30min; 94°C, 2min; 40 × (94°C, 30sec; 56°C, 30sec; 68°C, 30sec) by using the SuperScript III One-Step RT-RCR-Kit (Invitrogen).

To recover full-length viral genome sequences, viral RNA segments were reverse transcribed and amplified with the Invitrogen Superscript III One-Step RT-PCR Kit (Thermo Fisher Scientific) with a universal Primer Set targeting conserved regions of all eight segments (*1*). Primers were adjusted to a stock concentration of 10pmol/µl. The RT-PCR reaction consisted of 12.5µl 2x reaction buffer, 1µl of each primer, 1µl of Superscript III RT / Platinum Taq enzyme mix, 3.5µl nuclease free water and 5µl RNA sample adding up to a total volume of 25µl. The RT-PCR cycler program consisted of an initial 45-minute period at 45°C followed by a 2-minute denaturation step at 94°C, then 5 cycles of 94°C for 30s, 45°C for 30s and 68°C for 3 minutes followed by 30 cycles of 94°C for 30s, 57°C for 30s and 68°C for 3 minutes. The final Elongation step was 68°C for 5 minutes.

Following this, Illumina compatible DNA libraries were prepared (ILMN DNA LP (M) Tagmentation Kit) and sequenced on an Illumina NextSeq 1000 benchtop sequencer. The quality of the reads was checked with FastQC (*2*) and fastp (*3*). The final AIV genome assembly was performed with IRMA (*4*).

Histopathological and immunohistochemical examination

Samples were collected from different organs and tissues including rhinencephalon, the cerebellum and other parts of the brain, fixed in 10% neutral buffered formalin, processed by routine methods, embedded in paraffin wax, sectioned (4µm), and stained with hematoxylin and eosin (H&E).

Immunohistochemistry was performed on paraffin-embedded tissue sections by using a murine monoclonal antibody specific for the nucleoprotein (*5*). Briefly, 3µm tissue sections were deparaffinized and rehydrated using a descending series of alcohol. Afterwards, slides were incubated in 85% ethanol with additional 0.5% H_2O_2 for 30 min. at room temperature (RT) for blocking endogenous peroxidase activities. Enzymatic pre-treatment with proteinase K was performed for 10min. at RT and unspecific background staining was blocked with inactivated goat serum diluted 1:5 in phosphate-buffered saline (PBS). The primary anti-influenza A antibody (CLONEGENE LLC, HB65) was diluted 1:200 in PBS with 1% bovine serum albumin and slides were incubated overnight at 4°C. For negative control, the primary antibody was substituted by ascites fluid from Balb/c mice. Biotinylated goat-anti-mouse IgG was used as secondary antibody and added for 45min at RT. For visualization of immunostaining, the avidin-biotin-peroxidase complex (VECTASTAIN Elite ABC Kit; Vector Laboratories, Burlingame, CA) was applied by using 3,3`-diaminobenzidine-tetrahydrochloride (Sigma Aldrich) as chromogen. Counterstaining was performed with Mayer`s hemalaun.

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Appendix Table 1. Overview of wild terrestrial carnivores examined from February 2022 to April 2023. The months in which animals tested positive are in bold type*

	Number of animals tested/number of AIV positive animals					
Year/month	Red fox	Racoon	Badger	Marten	Racoon dog	Total
2022/Feb	11/0	2/0	3/0	0/0	1/0	17/0
2022/Mar	17/0	2/0	0/0	1/0	0/0	20/0
2022/Apr	2/0	0/0	0/0	1/0	0/0	3/0
2022/May	7/0	0/0	1/0	0/0	0/0	8/0
2022/Jun	6/0	0/0	0/0	1/0	0/0	7/0
2022/Jul	4/0	3/0	2/0	1/0	0/0	10/0
2022/Aug	3/0	1/0	2/0	0/0	1/0	7/0
2022/Sep	4/0	3/0	0/0	0/0	0/0	7/0
2022/Oct	8/0	9/0	2/0	0/0	1/0	20/0
2022/Nov	5/0	1/0	3/0	0/0	2/0	11/0
2022/Dec	10/0	5/0	0/0	1/0	1/0	17/0
2023/Jan	**9/3**	0/0	2/0	0/0	0/0	**11/3**
2023/Feb	**9/1**	0/0	0/0	1/0	2/0	**12/1**
2023/Mar	**12/1**	1/0	0/0	2/0	0/0	**15/1**
2023/Apr	3/0	1/0	0/0	1/0	0/0	5/0
Total	110/5	28/0	15/0	9/0	8/0	170/5

*Months in which animals tested positive are in bold type.

Appendix Table 2. Isolates used in this study*

Isolate ID	Country	Collection date	Isolate name	Originating Laoratory	Submitting Laboratory	Authors
EPI_ISL_17521049	United Kingdom	2023-Mar-23	A/black-headed_gull/England/053469/2023	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	
EPI_ISL_17470105	Czech Republic	2023-Apr-04	A/black-headed_gull/Czech_Republic/5152–1/2023	State Veterinary Institute Prague	State Veterinary Institute Prague	Alexander,Nagy;Lenka,Cernikova;Martina,Stara
EPI_ISL_17267144	Netherlands	2023-Mar-01	A/Black-headed gull/Netherlands/15/2023	Erasmus Medical Center	Erasmus Medical Center	
EPI_ISL_17606758	Belgium	2023-Mar-29	A/Vulpes_vulpes/Belgium/03117_0004/2023	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Roupie, Virginie; Hostyn, Pierre; Mathijs, Elisabeth; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_17245099	Belgium	2023-Feb-27	A/Vulpes_vulpes/Belgium/01784_0010/2023	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Roupie, Virginie; Hostyn, Pierre; Mathijs, Elisabeth; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_17244973	Belgium	2023-Feb-27	A/Vulpes_vulpes/Belgium/01784_0004/2023	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Roupie, Virginie; Hostyn, Pierre; Mathijs, Elisabeth; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_14494796	Belgium	2022-Jun-16	A/Vulpes_vulpes/Belgium/9031_0008/2022	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Mathijs, Elisabeth; Roupie, Virginie; Vervaeke, Muriel; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_15878539	Spain	2022-Oct-18	A/Mink/Spain/3691–8_22VIR10586–10/2022	Laboratorio Central de Veterinaria	Istituto Zooprofilattico Sperimentale delle Venezie	Ruano, M.J.; Rocha, A.; Sanchez, A.; Aguero, M; Barbierato, G.; Zecchin, B.; Fusaro, A.; Schivo, A.; Salviato, A.; Palumbo, E.; Giussani, E.; Pastori, A.; Monne, I.; Terregino, C.
EPI_ISL_15878542	Spain	2022-Oct-18	A/Mink/Spain/3691–3_22VIR10586–9/2022	Laboratorio Central de Veterinaria	Istituto Zooprofilattico Sperimentale delle Venezie	Ruano, M.J.; Rocha, A.; Sanchez, A.; Aguero, M; Barbierato, G.; Zecchin, B.; Fusaro, A.; Schivo, A.; Salviato, A.; Palumbo, E.; Giussani, E.; Pastori, A.; Monne, I.; Terregino, C.
EPI_ISL_12069289	Netherlands	2022-Jan-22	A/Fox/Netherlands/EMC3/2022	Erasmus Medical Center	Erasmus Medical Center	R.A.M., Fouchier; S., Thewessen; O., Vuong; I., Chestakova; R., Sikkema; P., Lexmond; M., Pronk
EPI_ISL_14571448	Germany	2022-May-03	A/great cormorant/Germany-MV/AI02666/2022	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF)	Friedrich-Loeffler-Institut	
EPI_ISL_14573037	Germany	2022-May-31	A/sandwich tern/Germany-MV/AI03131/2022	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF)	Friedrich-Loeffler-Institut	

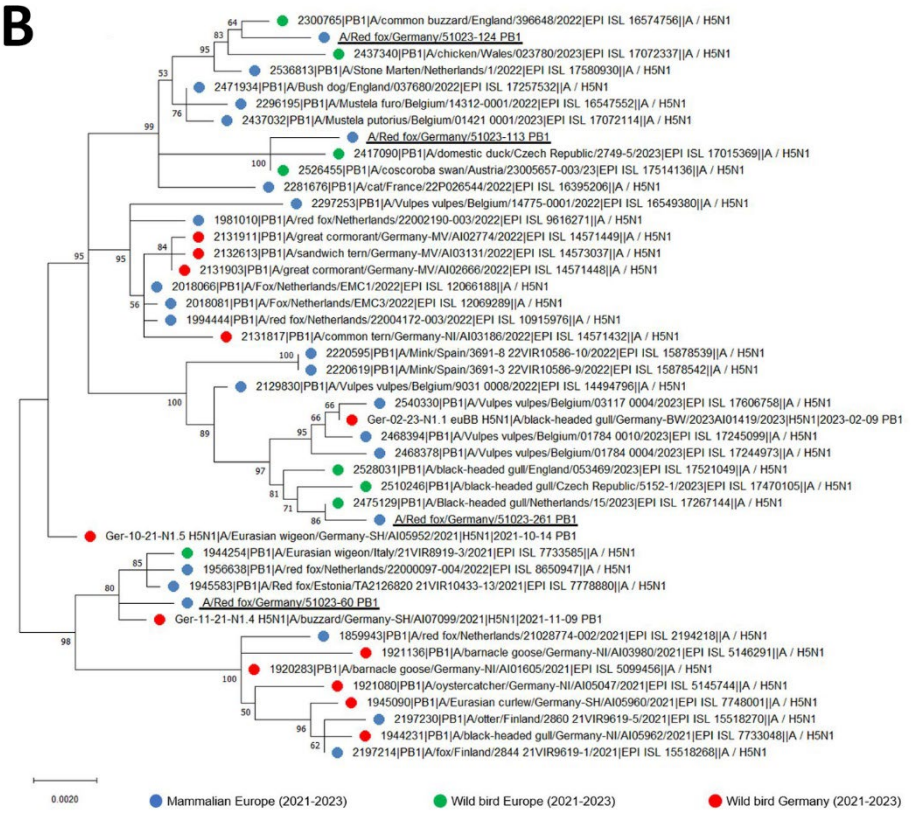
Isolate ID	Country	Collection date	Isolate name	Originating Laoratory	Submitting Laboratory	Authors
EPI_ISL_16549380	Belgium	2022-Dec-08	A/Vulpes_vulpes/Belgium/14775–0001/2022	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Roupie, Virginie; Hostyn, Pierre; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_12066188	Netherlands	2022-Jan-26	A/Fox/Netherlands/EMC1/2022	Erasmus Medical Center	Erasmus Medical Center	R.A.M., Fouchier; S., Therwessen; O., Vuong; I., Chestakova; R., Sikkema; P., Lexmond; M., Pronk
EPI_ISL_9616271	Netherlands	2022-Jan-31	A/red fox/Netherlands/22002190–003/2022	Wageningen Bioveterinary Research	Wageningen Bioveterinary Research	Beerens, Nancy; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Venema, Sandra; Germeraad, Evelien; Engelsma, Marc; Heutink, Rene
EPI_ISL_10915976	Netherlands	2022-Mar-01	A/red fox/Netherlands/22004172–003/2022	Wageningen Bioveterinary Research	Wageningen Bioveterinary Research	Beerens, Nancy; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Venema, Sandra; Germeraad, Evelien; Engelsma, Marc; Heutink, Rene
EPI_ISL_14571449	Germany	2022-May-06	A/great cormorant/Germany-MV/AI02774/2022	Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF)	Friedrich-Loeffler-Institut	
EPI_ISL_14571432	Germany	2022-Jun-07	A/common tern/Germany-NI/AI03186/2022	Lebensmittel- und Veterinärinstitut Oldenburg - Standort Veterinärinstitut	Friedrich-Loeffler-Institut	
EPI_ISL_17580930	Netherlands	2022-Jan-16	A/Stone Marten/Netherlands/1/2022	Erasmus Medical Center	Erasmus Medical Center	Vuong,O;Thewessen, S;Bellido-Martin,A.B; Chestakova, I; Sikkema, R;Fouchier, R.A.M.
EPI_ISL_17583228	Netherlands	2022-Sep-13	A/Fox/Netherlands/EMC7/2022	Erasmus Medical Center	Erasmus Medical Center	Vuong,O;Thewessen, S;Bellido-Martin,A.B; Chestakova, I; Sikkema, R;Fouchier, R.A.M.
EPI_ISL_17072337	United Kingdom	2023-Feb-11	A/chicken/Wales/023780/2023	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	
EPI_ISL_16574756	United Kingdom	2022-Dec-20	A/common_buzzard/England/396648/2022	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	
EPI_ISL_17015369	Czech Republic	2023-Feb-16	A/domestic_duck/Czech_Republic/2749–5/2023	State Veterinary Institute Prague	State Veterinary Institute Prague	Alexander,Nagy;Lenka,Cernikova;Martina,Stara
EPI_ISL_17514136	Austria	2023-Jan-18	A/coscoroba swan/Austria/23005657–003/23	Institute for Veterinary Disease Control Moedling, Austrian Agency for Health and Food Safety	Austrian Agency for Health and Food Safety (AGES)	

Isolate ID	Country	Collection date	Isolate name	Originating Laoratory	Submitting Laboratory	Authors
EPI_ISL_16395206	France	2022-Dec-24	A/cat/France/22P026544/2022	Anses (Ploufragan-Plouzané)	ANSES Agence Nationale De Securite Sanitaire De L'alimentation	
EPI_ISL_17257532	United Kingdom	2022-Nov-18	A/Bush_dog/England/037680/2022	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	
EPI_ISL_16547552	Belgium	2022-Dec-08	A/Mustela_furo/Belgium/14312–0001/2022	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Roupie, Virginie; Hostyn, Pierre; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_17072114	Belgium	2023-Feb-09	A/Mustela_putorius/Belgium/01421_0001/2023	Sciensano - Animal Infectious Diseases	Sciensano, Department of Animal Infectious Diseases	Van Borm, Steven; Roupie, Virginie; Hostyn, Pierre; Mathijs, Elisabeth; Lambrecht, Benedicte; Steensels, Mieke
EPI_ISL_7778880	Estonia	2021-Nov-08	A/Red_fox/Estonia/TA2126820_21VIR10433–13/2021	Estonian Veterinary and Food Laboratory	Istituto Zooprofilattico Sperimentale Delle Venezie	Nurmoja, I.; Vilem, A.; Juurik, T.; Zecchin, B.; Fusaro, A.; Schivo, A.; Salviato, A.; Palumbo, E.; Milani, A.; Giussani, E.; Pastori, A.; Monne, I.; Terregino, C.
EPI_ISL_11725994	Germany	2021-Nov-09	A/buzzard/Germany-SH/AI07099/2021	Landeslabor Schleswig-Holstein	Friedrich-Loeffler-Institut	
EPI_ISL_8650947	Netherlands	2022-Jan-03	A/red fox/Netherlands/22000097–004/2022	Wageningen Bioveterinary Research	Wageningen Bioveterinary Research	Beerens, Nancy; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Venema, Sandra; Germeraad, Evelien; Engelsma, Marc; Heutink, Rene
EPI_ISL_16618971	Russian Federation	2022-Oct-12	A/domestic goose/Magadan/14–9V/2022	State Research Center of Virology and Biotechnology (VECTOR)	State Research Center of Virology and Biotechnology (VECTOR)	
EPI_ISL_13692635	Russian Federation	2022-Apr-21	A/crow/Khabarovsk/216–13V/2022	State Research Center of Virology and Biotechnology (VECTOR)	State Research Center of Virology and Biotechnology (VECTOR)	
EPI_ISL_5146291	Germany	2021-Apr-14	A/barnacle goose/Germany-NI/AI03980/2021	Lebensmittel- und Veterinärinstitut Oldenburg - Standort Veterinärinstitut	Friedrich-Loeffler-Institut	
EPI_ISL_2194218	Netherlands	2021-May-17	A/red fox/Netherlands/21028774–002/2021	Wageningen Bioveterinary Research	Wageningen Bioveterinary Research	Beerens, Nancy; Harders, Frank; Pritz-Verschuren, Sylvia; Roose, Marit; Germeraad, Evelien; Engelsma, Marc; Bossers, Alex; Heutink, Rene
EPI_ISL_5099456	Germany	2021-Feb-17	A/barnacle goose/Germany-NI/AI01605/2021	Lebensmittel- und Veterinärinstitut Oldenburg - Standort Veterinärinstitut	Friedrich-Loeffler-Institut	

Isolate ID	Country	Collection date	Isolate name	Originating Laoratory	Submitting Laboratory	Authors
EPI_ISL_5145744	Germany	2021-Jul-17	A/oystercatcher/Germany-NI/AI05047/2021	Lebensmittel- und Veterinärinstitut Oldenburg - Standort Veterinärinstitut	Friedrich-Loeffler-Institut	
EPI_ISL_15518268	Finland	2021-Sep-01	A/fox/Finland/2844_21VIR9619–1/2021	Finnish Food Authority	Istituto Zooprofilattico Sperimentale Delle Venezie	Tammiranta, N.;Kantala, T.;Laamanen, I.;Gadd, T.;Zecchin, B.;Fusaro, A.;Schivo, A.;Salviato, A.;Palumbo, E.;Giussani, E.;Pastori, A.;Monne, I.;Terregino, C.
EPI_ISL_7733048	Germany	2021-Oct-12	A/black-headed gull/Germany-NI/AI05962/2021	Lebensmittel- und Veterinärinstitut Oldenburg - Standort Veterinärinstitut	Friedrich-Loeffler-Institut	
EPI_ISL_15518270	Finland	2021-Sep-01	A/otter/Finland/2860_21VIR9619–5/2021	Finnish Food Authority	Istituto Zooprofilattico Sperimentale Delle Venezie	Tammiranta, N.;Kantala, T.;Laamanen, I.;Gadd, T.;Zecchin, B.;Fusaro, A.;Schivo, A.;Salviato, A.;Palumbo, E.;Milani, A.;Giussani, E.;Pastori, A.;Monne, I.;Terregino, C.
EPI_ISL_7748001	Germany	2021-Oct-14	A/Eurasian curlew/Germany-SH/AI05960/2021	Landeslabor Schleswig-Holstein	Friedrich-Loeffler-Institut	
EPI_ISL_7733585	Italy	2021-Oct-28	A/Eurasian_wigeon/Italy/21VIR8919–3/2021	Istituto Zooprofilattico Speriment. Lombardia, Emilia Ro. IZSLER	Istituto Zooprofilattico Sperimentale Delle Venezie	Zecchin, B.; Fusaro, A.; Schivo, A.; Salviato, A.; Palumbo, E.; Milani, A.; Giussani, E.; Pastori, A.; Monne, I.; Terregino, C.
EPI_ISL_17880904	Germany	2023-Jan-09	A/Red fox/Germany/51023–60/2023	Lower Saxony State Office for Consumer Protection and Food Safety	Lower Saxony State Office for Consumer Protection and Food Safety	This study
EPI_ISL_17880908	Germany	2023-Jan-31	A/Red fox/Germany/51023–113/2023	Lower Saxony State Office for Consumer Protection and Food Safety	Lower Saxony State Office for Consumer Protection and Food Safety	This study
EPI_ISL_17880911	Germany	2023-Feb-09	A/Red fox/Germany/51023–124/2023	Lower Saxony State Office for Consumer Protection and Food Safety	Lower Saxony State Office for Consumer Protection and Food Safety	This study
EPI_ISL_17880914	Germany	2023-Mar-29	A/Red fox/Germany/51023–261/2023	Lower Saxony State Office for Consumer Protection and Food Safety	Lower Saxony State Office for Consumer Protection and Food Safety	This study

*We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database on which this research is based. All submitters of data may be contacted directly via www.gisaid.org. Sequences obtained in this study are listed at the end of this table.


A
2437033|PB2|A/Mustela putorius/Belgium/01421 0001/2023|EPI ISL 17072114||A / H5N1
2471933|PB2|A/Bush dog/England/037680/2022|EPI ISL 17257532||A / H5N1
2281675|PB2|A/cat/France/22P026544/2022|EPI ISL 16395206||A / H5N1
A/Red fox/Germany/51023-113 PB2
2417089|PB2|A/domestic duck/Czech Republic/2749-5/2023|EPI ISL 17015369||A / H5N1
2526454|PB2|A/coscoroba swan/Austria/23005657-003/23|EPI ISL 17514136||A / H5N1
2296194|PB2|A/Mustela furo/Belgium/14312-0001/2022|EPI ISL 16547552||A / H5N1
2536812|PB2|A/Stone Marten/Netherlands/1/2022|EPI ISL 17580930||A / H5N1
2437339|PB2|A/chicken/Wales/023780/2023|EPI ISL 17072337||A / H5N1
2536836|PB2|A/Fox/Netherlands/EMC7/2022|EPI ISL 17583228||A / H5N1
2300763|PB2|A/common buzzard/England/396648/2022|EPI ISL 16574756||A / H5N1
A/Red fox/Germany/51023-124 PB2
1994443|PB2|A/red fox/Netherlands/22004172-003/2022|EPI ISL 10915976||A / H5N1
2018080|PB2|A/Fox/Netherlands/EMC3/2022|EPI ISL 12069289||A / H5N1
2131818|PB2|A/common tern/Germany-NI/AI03186/2022|EPI ISL 14571432||A / H5N1
2297254|PB2|A/Vulpes vulpes/Belgium/14775-0001/2022|EPI ISL 16549380||A / H5N1
1981009|PB2|A/red fox/Netherlands/22002190-003/2022|EPI ISL 9616271||A / H5N1
2018065|PB2|A/Fox/Netherlands/EMC1/2022|EPI ISL 12066188||A / H5N1
2132614|PB2|A/sandwich tern/Germany-MV/AI03131/2022|EPI ISL 14573037||A / H5N1
2131904|PB2|A/great cormorant/Germany-MV/AI02666/2022|EPI ISL 14571448||A / H5N1
2131912|PB2|A/great cormorant/Germany-MV/AI02774/2022|EPI ISL 14571449||A / H5N1
2220594|PB2|A/Mink/Spain/3691-8 22VIR10586-10/2022|EPI ISL 15878539||A / H5N1
2220618|PB2|A/Mink/Spain/3691-3 22VIR10586-9/2022|EPI ISL 15878542||A / H5N1
2129829|PB2|A/Vulpes vulpes/Belgium/9031 0008/2022|EPI ISL 14494796||A / H5N1
2468377|PB2|A/Vulpes vulpes/Belgium/01784 0004/2023|EPI ISL 17244973||A / H5N1
Ger-02-23-N1.1 euBB H5N1|A/black-headed gull/Germany-BW/2023AI01419/2023|H5N1|2023-02-09 PB2
2540329|PB2|A/Vulpes vulpes/Belgium/03117 0004/2023|EPI ISL 17606758||A / H5N1
2468393|PB2|A/Vulpes vulpes/Belgium/01784 0010/2023|EPI ISL 17245099||A / H5N1
2528030|PB2|A/black-headed gull/England/053469/2023|EPI ISL 17521049||A / H5N1
A/Red fox/Germany/51023-261 PB2
2475128|PB2|A/Black-headed gull/Netherlands/15/2023|EPI ISL 17267144||A / H5N1
2510245|PB2|A/black-headed gull/Czech Republic/5152-1/2023|EPI ISL 17470105||A / H5N1
Ger-10-21-N1.5 H5N1|A/Eurasian wigeon/Germany-SH/AI05952/2021|H5N1|2021-10-14 PB2
1945582|PB2|A/Red fox/Estonia/TA2126820 21VIR10433-13/2021|EPI ISL 7778880||A / H5N1
Ger-11-21-N1.4 H5N1|A/buzzard/Germany-SH/AI07099/2021|H5N1|2021-11-09 PB2
1944253|PB2|A/Eurasian wigeon/Italy/21VIR8919-3/2021|EPI ISL 7733585||A / H5N1
1956637|PB2|A/red fox/Netherlands/22000097-004/2022|EPI ISL 8650947||A / H5N1
A/Red fox/Germany/51023-60 PB2
1921137|PB2|A/barnacle goose/Germany-NI/AI03980/2021|EPI ISL 5146291||A / H5N1
1921081|PB2|A/oystercatcher/Germany-NI/AI05047/2021|EPI ISL 5145744||A / H5N1
1920284|PB2|A/barnacle goose/Germany-NI/AI01605/2021|EPI ISL 5099456||A / H5N1
1859942|PB2|A/red fox/Netherlands/21028774-002/2021|EPI ISL 2194218||A / H5N1
1945091|PB2|A/Eurasian curlew/Germany-SH/AI05960/2021|EPI ISL 7748001||A / H5N1
1944232|PB2|A/black-headed gull/Germany-NI/AI05962/2021|EPI ISL 7733048||A / H5N1
2197213|PB2|A/fox/Finland/2844 21VIR9619-1/2021|EPI ISL 15518268||A / H5N1
2197229|PB2|A/otter/Finland/2860 21VIR9619-5/2021|EPI ISL 15518270||A / H5N1
0.01
Mammalian Europe (2021-2023)
Wild bird Europe (2021-2023)
Wild bird Germany (2021-2023)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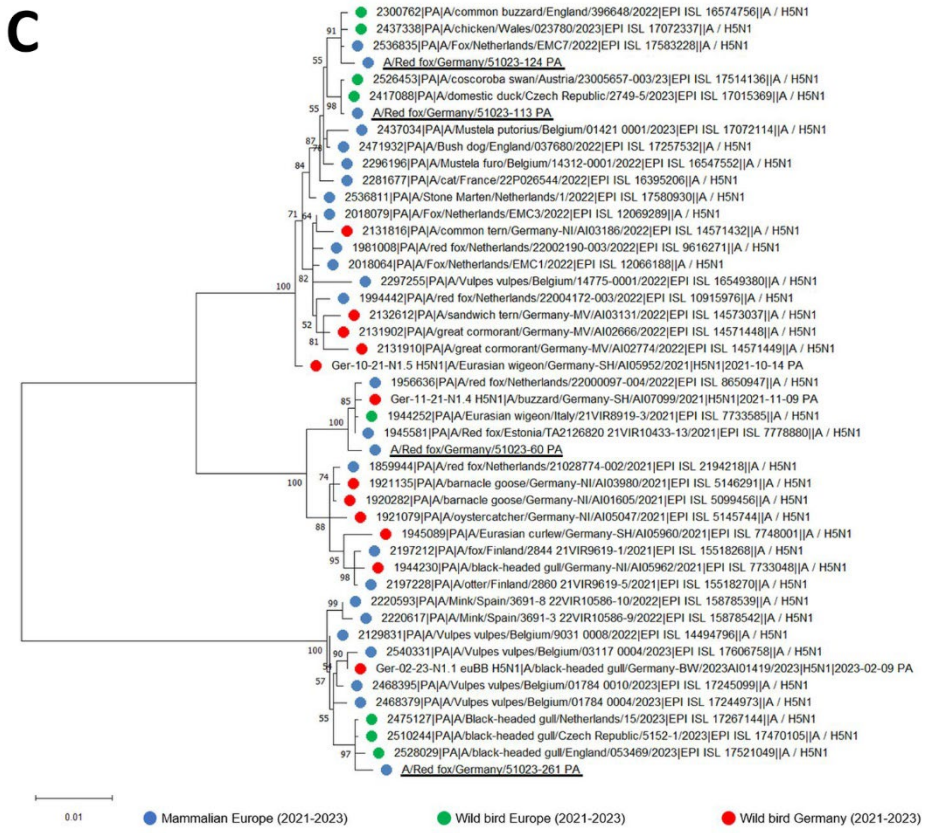
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Wild bird Europe (2021-2023)
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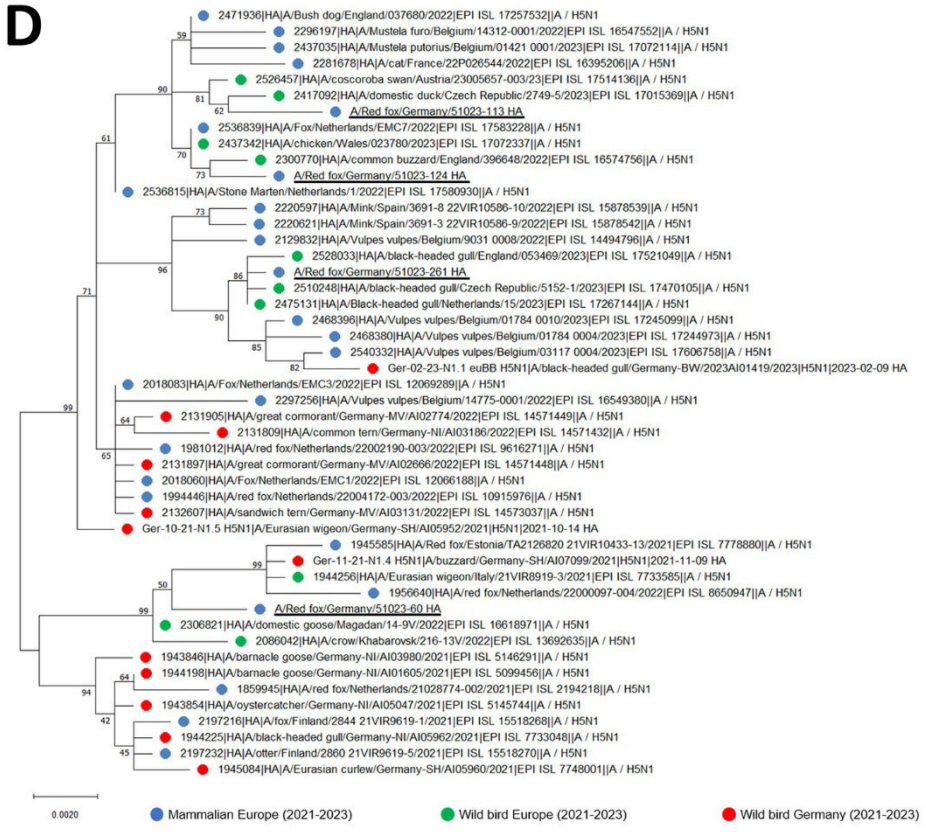
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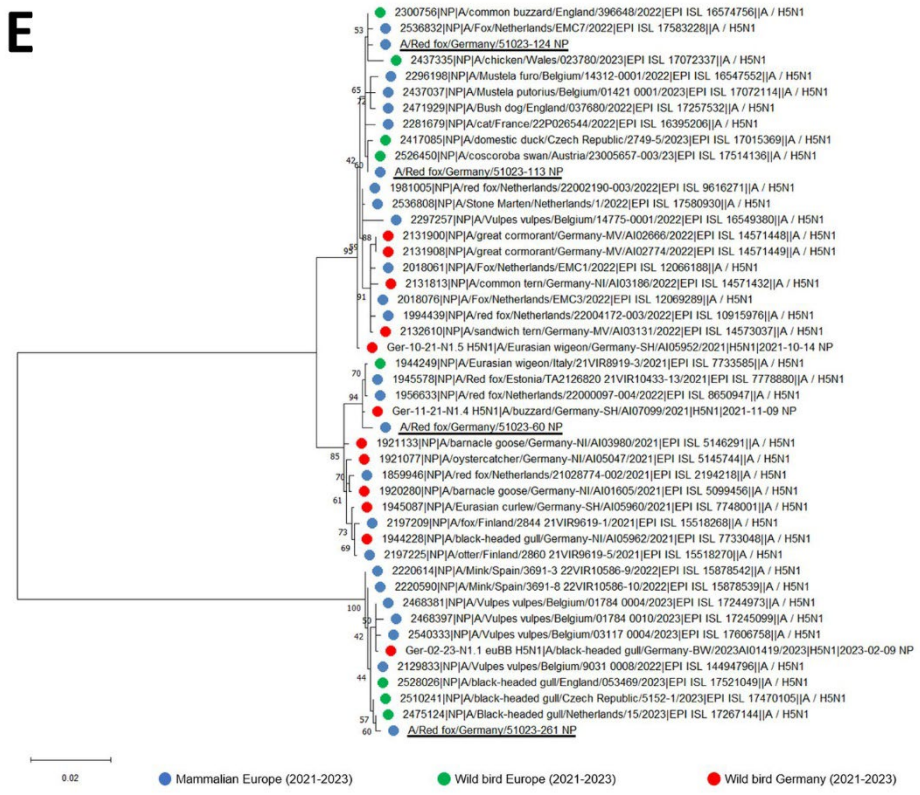
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Wild bird Europe (2021-2023)
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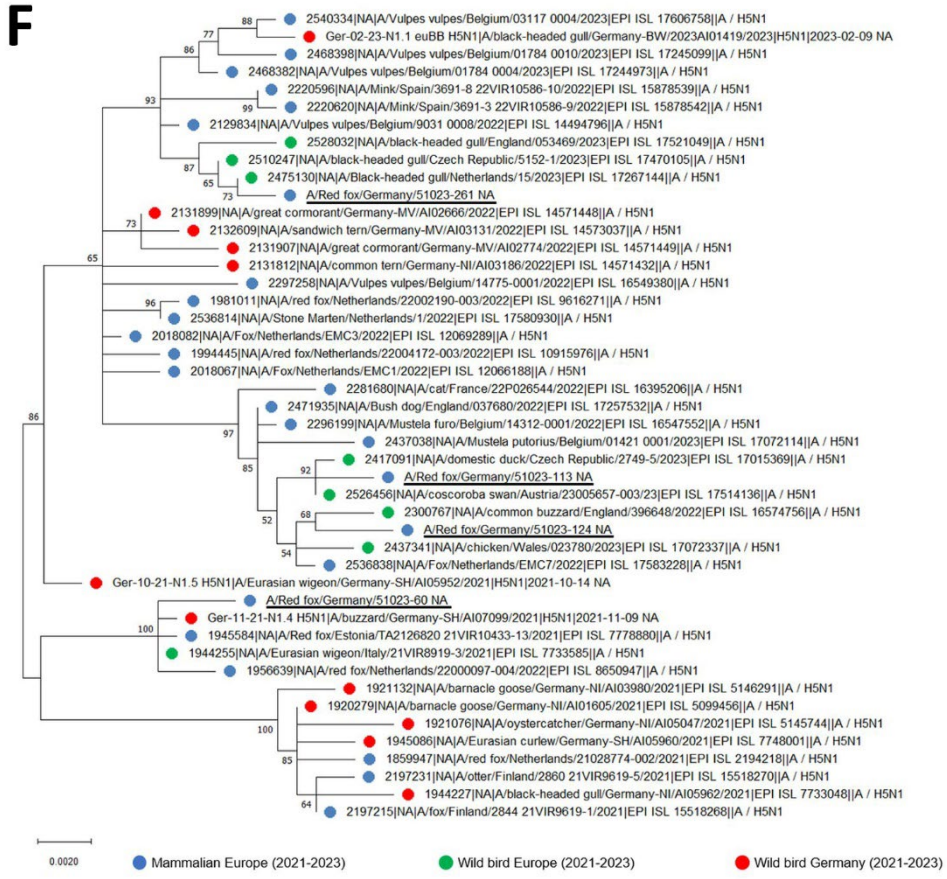
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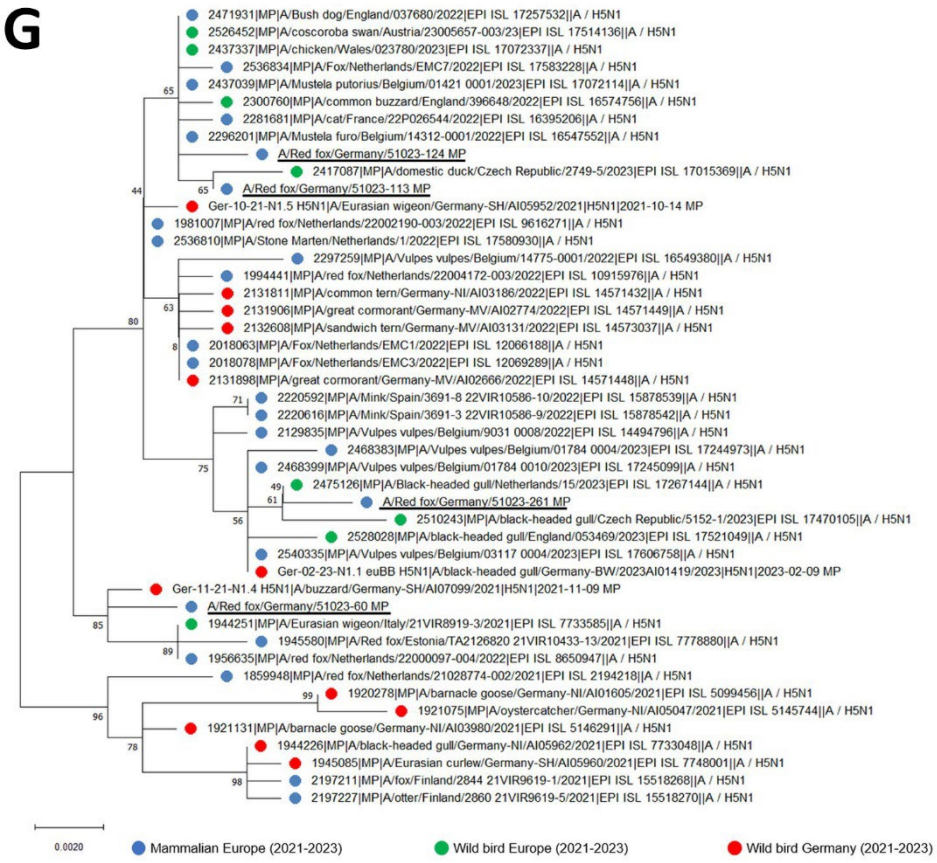
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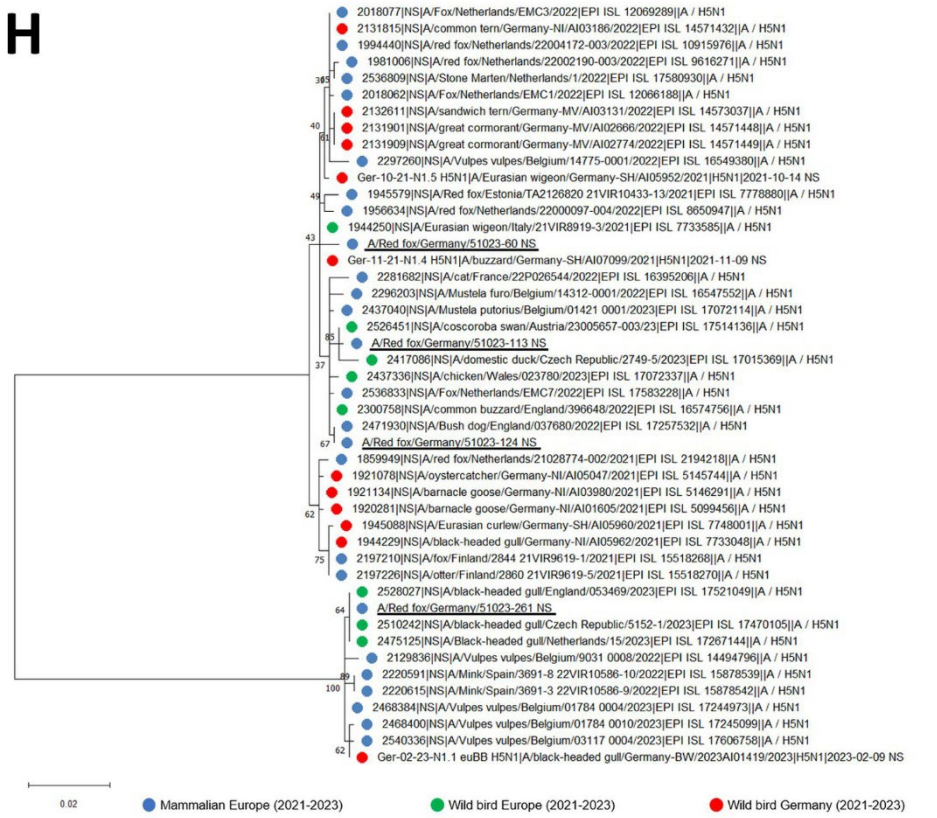
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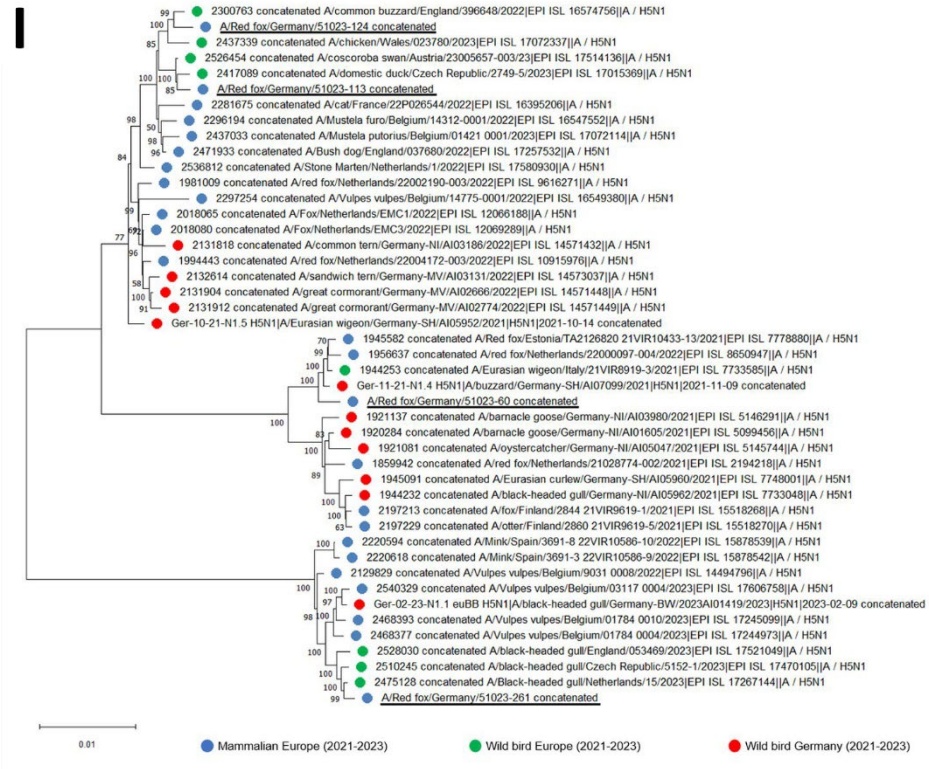

Appendix Figure. Phylogenetic maximum-likelihood analyses of all 8 HPAIV H5 segmental sequences and concatenated genomes of mammals and wild birds with 500 bootstrap iterations. Virus variants found in this study are underlined. Numbers along branches indicate percentage bootstrap values. Scale bars indicate nucleotide substitutions per site. A) polymerase basic 2; B) polymerase basic 1; C) polymerase acidic; D) hemagglutinin; E) nucleoprotein; F) neuraminidase; G) matrix; H, nonstructural protein; I) concatenated.