Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus in Poultry, Benin, 2021

Appendix

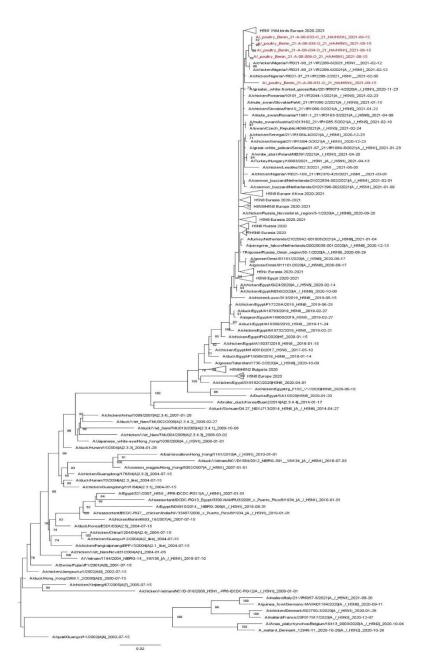
		A/poultry/Benin/21-A-08-009-O/2021, A/poultry/Benin/21-A-09-033-O/2021,
	A/poultry/Benin/21-A-09-031-O/2021,	A/poultry/Benin/21-A-09-034-O/2021, A/poultry/Benin/21-A-09-035-O/2021
Segment	tMRCA (95% HPD range)†	tMRCA (95% HPD range)†
PB2	Apr (Mar–May)	Aug (Aug–Oct)
PB1	Mar (Jan–Jul)	Jul (Mar–Sep)
PA	Apr (Feb–May)	Aug (Jun–Oct)
HA	Mar (Feb–May)	Sep (May–Oct)
NP	Mar (Feb–May)	Jul (Mar–Aug)
NA	Apr (Feb–May)	Jul (Apr–Sep)
M	Aug (May–Oct)	Aug (May–Oct)
NS	Apr (Feb–May)	Aug (May–Oct)

neuraminidase; M, matrix protein; NS, nonstructural protein; tMRCA, time to most recent common ancestor; HPD, highest posterior density. †All dates 2020

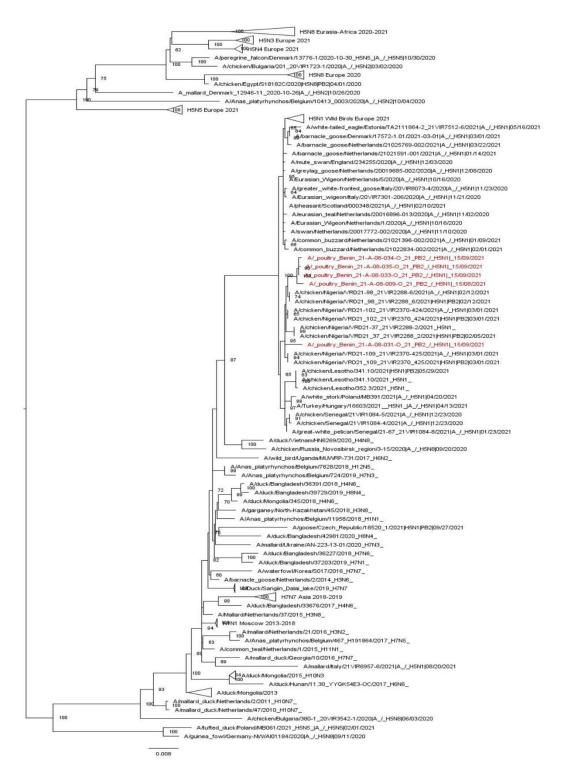
	Appendix Table 2. Hemagglutination inhibition assa	y of clade 2.3.4.4 viruses	(with 0.5% turke	y red blood cells)	
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			Sichuan	Fujian-S	Astrakhan		Hubei		Guang
Virus	Subtype	Clade	/14	·/1	/20*	Gyr/Wa	/16	Dk/Hyogo	/18
Reference antigen									
IDCDC-RG42A	H5N6	2.3.4.4a	320	160	160	40	10	640	<10
(A/Sichuan/26221/2014-like)									
A/Fujian-Sanyuan/21099/2017	H5N6	2.3.4.4b	160	160	160	80	0	320	<10
(CNIC-21099)									
CBER-RG8A	H5N6	2.3.4.4b	160	160	320	160	<10	320	<10
(A/Astrakhan/3212/2020-like)									
IDCDC-RG43A	H5N6	2.3.4.4c	160	160	160	80	<10	320	<10
(A/gyrfalcon/WA/41088-6/2014-like)									
A/Hubei/29578/2016 (CNIC-29578)	H5N6	2.3.4.4d	<10	<10	<10	<10	160	10	10
A/duck/Hyogo/1/2016 (NIID-001)	H5N6	2.3.4.4e	320	320	320	320	10	640	<10
IDCDC-RG-65A	H5N6	2.3.4.4f	<10	<10	<10	<10	10	10	160
(A/Guangdong/18SF020/2018-like)									
Test antigen									
A/poultry/Benin/21-A-08-009-O/2021	H5N1	2.3.4.4b	<10	10	40†	10	<10	40	<10
A/poultry/Benin/21-A-09-031-O/2021	H5N1	2.3.4.4b	640	320	320‡	640	<10	640	<10
A/poultry/Benin/21-A-09-033-O/2021	H5N1	2.3.4.4b	<10	10	40†	10	<10	40	<10
A/poultry/Benin/21-A-09-034-O/2021	H5N1	2.3.4.4b	<10	10	80§	10	<10	40	<10
A/poultry/Benin/21-A-09-035-O/2021	H5N1	2.3.4.4b	<10	20	160‡	20	<10	160	<10
*Closest candidate viruses									

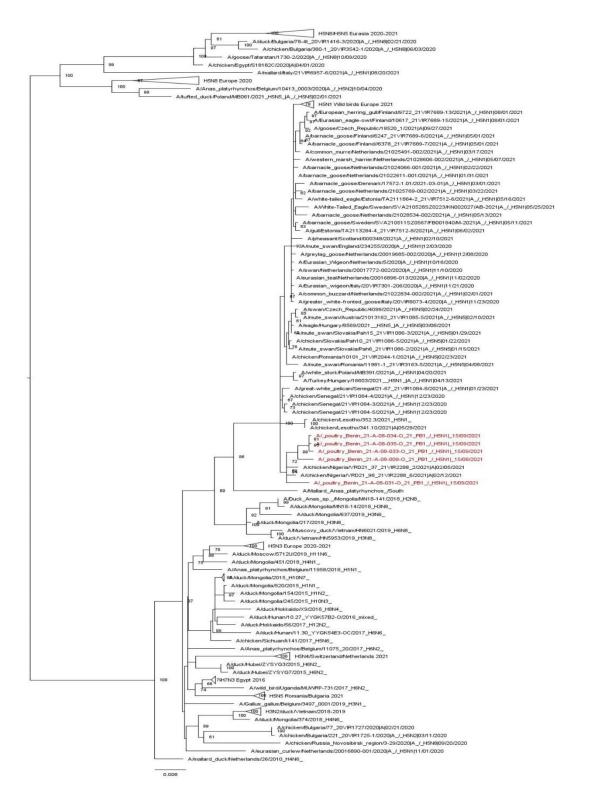
†8 fold <u>‡≤</u>2 fold §4 fold



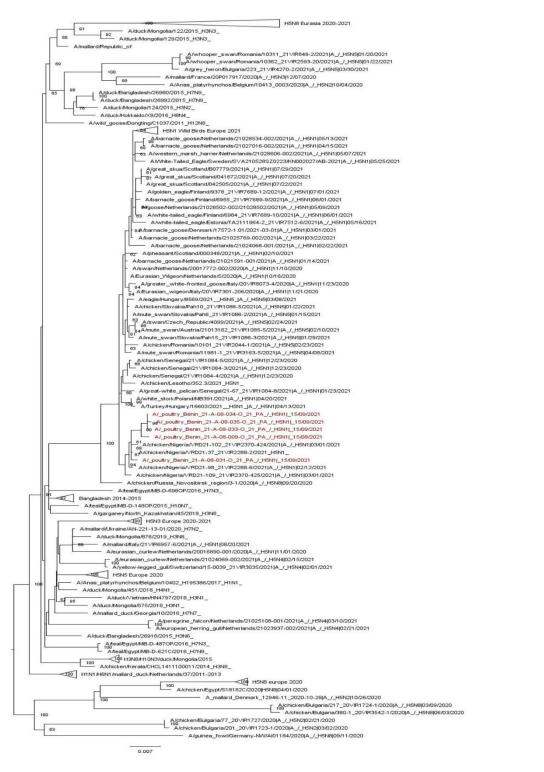
Appendix Figure 1. Maximum likelihood phylogenetic tree of the hemagglutinin (HA) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.



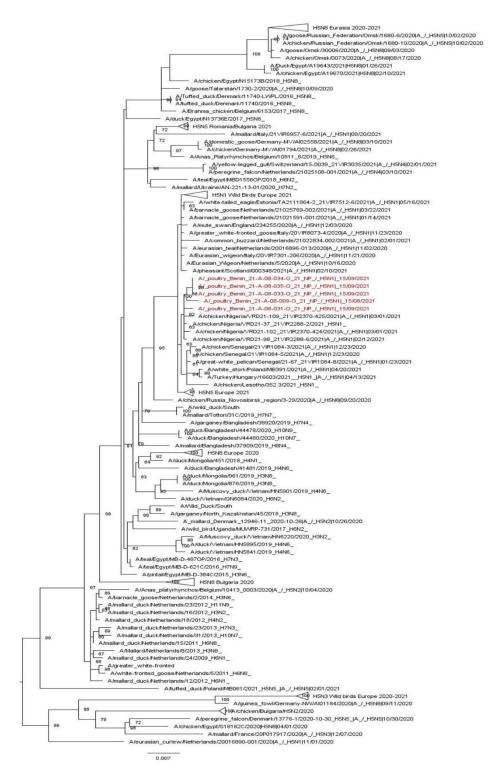
Appendix Figure 2. Maximum Likelihood phylogenetic tree of the polymerase basic 2 (PB2) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.



Appendix Figure 3. Maximum likelihood phylogenetic tree of the polymerase basic 1 (PB1) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values \geq 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.



Appendix Figure 4. Maximum likelihood phylogenetic tree of the polymerase acidic (PA) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.



Appendix Figure 5. Maximum likelihood phylogenetic tree of the nucleoprotein (NP) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.



Appendix Figure 6. Maximum likelihood phylogenetic tree of the neuraminidase (NA) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.



Appendix Figure 7. Maximum likelihood phylogenetic tree of the matrix protein (M) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.

	H5N8 Eurasia Africa 2020-2021
	A/eurasian_curlew/Netherlands/21024069-002/2021/A_/_H5N4[02/15/2021 Wperegrine_falcon/Netherlands/21025108-001/2021/A_/_H5N4[03/10/2021
	A/yellow-legged_gull/Switzerland/15-0039_21VIR3035/2021 A_/_H5N4[02/01/2021
	A/whooper_swan/Romania/10311_21VIR849-2/2021 A_/_H5N5/01/20/2021
	Wwhooper_swan/Romania/10213_21VIR2593-27/2021/A_/_H5N5/01/14/2021
Ĩ	A/grey_heron/Bulgaria/223_21VIR4270-2/2021 [A_/_H5N5]03/30/2021 A/mailard/Netherlands/32/2014_mixed_
	A/duck/Moscow/4298/2010_H3N8_
	A/Mallard/Netherlands/31/2014_H4N3_
L. L	A/mallard_duck/Netherlands/1/2012_H10N7_
	H5N1 Wild birds 2021
	A/chicken/Senegal/21VIR1084-3/2021 A_/_H5N1 1 2/23/2020
	 A/chicken/Senegal/21VIR1084-5/2021[A_/_H5N1]12/23/2020 A/mute_swan/England/234255/2020[A_/_H5N1]12/03/2020
	A/pheasant/Scotland/000348/2021 JA_/_H5N1 J02/10/2021
	A/barnacle_goose/Netherlands/21027016-002/2021 [A_/_H5N1]04/15/2021
	A/barnacle_goose/Netherlands/21028534-002/2021 A_/_H5N1 05/13/2021
	A/Turkey/Hungary/16603/2021H5N1_IA_/_H5N1J04/13/2021
	L A/white_stork/Poland/MB391/2021 A_/_H5N1 04/20/2021 A/eurasian_teal/Netherlands/20016896-013/2020 A_/_H5N1 11/02/2020
	A/European_herring_gull/Finland/9722_21VIR7689-13/2021 [A_/_H5N1]08/01/2021
	WEurasian_eagle-owl/Finland/10617_21VIR7689-15/2021 A_/_H5N1 08/01/2021
	Algoose/Czech_Republic/18520_2/2021]H5N1[09/27/2021
	 A/gull/Estonia/TA2113284-4_21VIR7512-8/2021/A_/_H5N1/06/02/2021 A/barnacle_goose/Finland/6247_21VIR7689-6/2021/A_/_H5N1/05/01/2021
	A/barnacle_goose/Finland//6378_21VIR7689-7/2021JA_/_H5N1J05/01/2021
	A/barnacle_goose/Netherlands/21022611-001/2021 A_/_H5N1 01/31/2021
	A/mute_swan/Austria/21013162_21VIR1085-5/2021 JA_/_H5N5J02/10/2021
	A/mute_swan/Slovakia/Pah6_21VIR1086-2/2021JA_/_H5N5J01/15/2021
	A/eagle/Hungary/8569/2021H5N5_JA_/_H5N5]03/08/2021
	 A/swan/Czech_Republic/4099/2021 A_/_H5N5[02/24/2021 A/chicken/Romania/10101_21VIR2044-1/2021 A_/_H5N5[02/23/2021
	 A/mute_swan/Romania/11981-1_21VIR3163-5/2021 A_/_H5N5 04/08/2021
	A/_poultry_Benin_21-A-08-033-0_21_NS_/_H5N1 _15/09/2021
	▲_poultry_Benin_21-A-08-034-0_21_NS_/_H5N1[_15/09/2021
	♣/_poultry_Benin_21-A-08-035-0_21_NS_/_H5N1[_15/09/2021
	&_poultry_Benin_21-A-08-009-O_21_NS_/_H5N1[_15/08/2021
	A/greater_white-fronted_goose/Italy/20VIR8073-4/2020[A_/_H5N1]11/23/2020
	A/chicken/Nigeria/VRD21-98_21VIR2288-6/2021 A_/_H5N1 02/12/2021 A/White-Tailed_Eagle/Sweden/SVA210528SZ0223/KN002027/AB-2021 A_/_H5N1 05/25/2021
	Avvnite- railed_Eagle/Sweden/SVA2105/8520223/KN00202//AB-2021/AH5N1[05/25/2021 A/bamacle_goose/Sweden/SVA210511SZ0567/FB001840/M-2021 AH5N1[05/11/2021
	A poultry Benin 21-A-08-031-0 21 NS / H5N1 15/09/2021
	A/great-white_pelican/Senegal/21-67_21VIR1084-8/2021[A_/_H5N1]01/23/2021
	A/white-tailed_eagle/Estonia/TA2111864-2_21VIR7512-6/2021 A_/_H5N1 05/16/2021
	A/chicken/Nigeria/VRD21-109_21VIR2370-425/2021 [A_/_H5N1]03/01/2021
	A/Eurasian_Wigeon/Netherlands/5/2020[A_/_H5N1]10/16/2020
	 A/barnacle_goose/Denmark/17572-1.01/2021-03-01 A_/_H6N1 03/01/2021 A/barnacle_goose/Netherlands/21025769-002/2021 A_/_H5N1 03/22/2021
	Alwestern_marsh_harrier/Netherlands/21028606-002/2021[A_/_H5N1[05/22/2021
	- A/chicken/Lesotho/352.3/2021_H5N1_
	A/chicken/Nigeria/VRD21-37_21VIR2288-2/2021_H5N1_
	A/common_buzzard/Netherlands/21022834-002/2021/A_/_H5N1/02/01/2021
	A/common_buzzard/Netherlands/21021396-002/2021JA_/_H5N1J01/09/2021
	7_mallard_Denmark_12946-11_2020-10-26[A_/_H5N2]10/26/2020
	VP H5N3 Europe 2020-2021 AAnas_platythynchos/Belgium/9074/2016_H9N2_
	A/Anas_platyrhynchos/Belgium/11242/2016_H7N7
	d %/Anas_platyrhynchos/Belgium/11127/2016_H2N3_
	A/mallard_duck/Netherlands/6/2015_H10N7_
	- A/Mallard/Netherlands/37/2015_H3N8_
	A/mallard_duck/Netherlands/41/2015_H5N1_
	 A/mallard_duck/Netherlands/52/2015_H5N6_ A/Anas_platyrhynchos/Belgium/227_H192329_2017/2016_H6N2_
	A/Anas_platymynchos/Belgium/227_H192329_2017/2016_H6N2_ A/mallard/Netherlands/21/2016_H3N2_
	A/mallard_duck/Netherlands/59/2015_H6N5_
	¹ Ä/mallard_duck/Netherlands/60/2015_H6N5_
	A/Anas_platyrhynchos/Belgium/10402_H195386/2017_H1N1_
	A/mallard_duck/Netherlands/8/2014_H7N5_
	 A/Anas_platyrhynchos/Belgium/10413_0003/2020 A_/_H5N2 10/04/2020 A/Anas_platyrhynchos/Belgium/11089/2016_H11N2_
	- A/Anas_platymynchos/Belgium/10/89/2016_H11N2_ - A/Anas_platymynchos/Belgium/10/49_47/2017_H5N3_
	A/sanderling/iceland/6743/2017_H2N5_
	A/mailard_duck/Netherlands/6/2012_H1N1_
	A/mallard_duck/Netherlands/1/2013_H7N7_
	- A/mailard_duck/Netherlands/24/2014_H7N3_
	A/mallard_duck/Netherlands/5/2013_H1N1_ A/mallard_duck/Netherlands/20/2012_H1N1_
	 A/mallard_duck/Netherlands/20/2012_H1N1_ A/mallard_duck/Netherlands/23/2013_H7N3_
	A/mailard_duck/Netherlands/23/2013_H/N3_
	Amailard_duck/Sweden/139899/2012_H15N5_
	A/mallard_duck/Netherlands/32/2013_H10N7_
	- A/mallard_duck/Netherlands/12/2011_H6N2_
	A/duck/Moscow/4641/2011_H4N6_ A/mallard/Moscow/4528/2011_H4N6_
	· Amailard/Moscow/4528/2011_H4N6_ · A/duck/Moscow/4652/2011_H4N6_
	A/duck/Moscow/4681/2011_H3N8_
	A/mallard/Moscow/4843/2012_H4N6_
	- A/mallard_duck/Netherlands/5/2012_H10N7_
	- A/mallard_duck/Netherlands/37/2011_H1N1_
	Agreater_white-fronted
	A/mallard_duck/Netherlands/24/2011_H2N3_ A/mallard/iceland/2394/2012_H11N9_
	A/glaucous_gull/lceland/4552/2015_H10N7_
	Alceland_guil/iceland/4266/2015_H10N7_
	Chicken/Bulgaria/201_20V/R1723-1/2020/A_/_H5N2/03/02/2020
	A/guinea_fowl/Germany-NW/Al01184/2020/A_/_H5N8/09/11/2020
	A/mallard/ltaly/21VIR6957-6/2021JA_/_H5N1J08/20/2021
0.05	

Appendix Figure 8. Maximum Likelihood phylogenetic tree of the Nonstructural protein (NS) gene. H5N1 virus sequences from Benin are indicated in red. Bootstrap values ≥ 60% are shown near the nodes. Scale bar indicates the average number of nucleotide substitutions per site.