

## Supplementary tables and figures for manuscript:

### Emergency and genetic characterization of Highly Pathogenic influenza A(H5N1) virus of clade 2.3.4.4 in Egypt, 2021-2022

**Supplementary table S1.** Primers and probes used for RT-PCR identification of viruses in collected samples.

ID	Primer sequences	Reference
<b>AIV-M-gene</b>	sep1: AGATGAGTCTTCTAACCGAGGTCG sep2: TGCAAAAACATCTTCAAGTCTCTG sep-probe: FAM-TCAGGCCCCCTCAAAGCCGA-TAMRA	<a href="#">[1]</a>
<b>AIV-H5 subtype</b>	H5LH1: ACATATGACTACCCACARTATTTCAG H5RH1: AGACCAGCTAYCATGATTGC H5PRO: FAM-TCWACAGTGGCGAGTTCCTAGCA-TAMRA	<a href="#">[2]</a>
<b>AIV-H6 subtype</b>	IAV-H6-1666F: CTTGGTGTGTATCAAATYCTTGC IAV-H6-1776R: CATTGARCCATTTGARCACATCCA IAV-H6-1693: FAM-TATAGTACGGTATCGAGCAGYCT-MGB	<a href="#">[3]</a>
<b>AIV-H9 subtype</b>	For: GGAAGAATTAATTATTATTGGTCGGTAC Rev: GCCACCTTTTTTCAGTCTGACATT H9 probe: FAM-AACCAGGCCAGACATTGCGAGTAAGATCC-TAMRA	<a href="#">[4]</a>
<b>AIV-N1 subtype</b>	N1 forward: TAYAACTCAAGGTTTGAGTCTGTYGCTTG N1 reverse: ATGTTRTTCCTCCAACCTCTTGATRGTGTC N1-Probe: FAM-TCAGCRAGTGCTTGCCATGATGGCA-TAMRA	<a href="#">[3]</a>
<b>AIV-N2 subtype</b>	FN2: TGGACAGGGAACAACACTAAA C RN2: ACAAGCCTCCCATCGTAAAT N2-Probe: TXRED-CAAAATGAAATGGAACACCCAACTCAT-BHQ23	<a href="#">[3]</a>
<b>AIV-N8 subtype</b>	N8-1296F: TCCATGYTTTGGGTTGARATGAT N8 1423R: GCTCCATCRTGCCAYGACCA N8 A354-Probe: FAM-TCHAGYAGCTCCATTGTRATGTGTGGAGT-TAMRA	<a href="#">[3]</a>
<b>IBV</b>	AIBV-fr: ATGCTCAACCTTGTCCCTAGCA AIBV-as: TCAAAGTGCAGATCATCACGT AIBV-TM: FAM-TTGAAGTAGAGTGACGCCCCAACTTCA-TAMRA	<a href="#">[5]</a>
<b>NDV</b>	F+4839: TCCGGAGGATACAAGGGTCT F-4939: AGCTGTTGCAACCCCAAG F+4894: FAM-AAGCGTTTCTGTCTCCTTCCTCCA-TAMRA	<a href="#">[6]</a>
<b>ILTV</b>	ILTVgCU771: CCTTGCGTTTGAATTTTCTGT ILTVgCL873: TTCGTGGGTTAGAGGTCTGT ILTVprobe817: FAM-CAGCTCGGTGACCCCATCTA-MGBNFQ	<a href="#">[7]</a>

<b>WNV</b>	WNV10668: CAGACCACGCTACGGCG WNV11770: CTAGGGCCGCGTGGG WNV10692: FAM-TCTGCGGAGAGTGCAGTCTGCGAT-TAMRA	<a href="#">[8]</a>
<b>IBDV</b>	F/AUS GU: TCACCG TCCTCAGCTTACCCACATC R/AUS GL: GGATTGGGATCAGCTCGAAGTTGC	<a href="#">[9]</a>

**Supplementary table S2.** Primers used in Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for sequencing.

FLI= Friedrich-Loeffler-Institut; NLQP= The National laboratory for quality control on poultry production; SEPRL= The Southeast Poultry Research Laboratory.

Primer ID	Nucleotide Sequence	Reference
PB2 Primers		
PB2-1FV2	5'GGAAAGCAGGTCAAATATATTCAATA 3'	FLI
PB2-F1361	5'ATTGATAATGTCATGGGGATG 3'	NLQP
PB2-1421R	5' GGT AAT AAT CCG ATC ATC CCC AT 3'	NLQP
PB2-F-1775	5'TAG TAC CAA AGG CTG CCA GA 3'	NLQP
PB2-1RV2	5'GGTCGTTTTTAAACAATTCGACAC 3'	FLI
PB2-R828	5'TCTCCAGCAGTGATGCCAGTG 3'	NLQP
PB1 Primers		
PB1-2FV3	5'AGCAGGCAAACCATTTGAATGG3'	FLI
PB1-2F -1100	5'AC ACA AAT ACC AGC AGA AAT GCT 3'	NLQP
PB1-F-1433	5'GCA AGC TAG TCG GAA TCA A 3'	NLQP
Pb1 R-867	5'CAC GAC GTT TGC CAA TTT AGC C 3'	NLQP
PB1-1230R	5'ATGTTGAACATGCCCATCATCAT3'	NLQP
PB1-R-2300	5'TTC ATG AAG GAC AAG CTA A 3'	NLQP
PA Primers		
PA -3FV2	5'AGC GAA AGC AGG TAC TGA TTC AAA A 3'	FLI
PA+734	5'AACCGAACGGCTGCATTGA 3'	NLQP
PA-1131F	5'ACT CGG TGA GAA CAT GGC ACC3'	NLQP
PA-F1825	5'ACATGACCAAAGAATTCTTTGA3'	NLQP
PA -1312R	5'CTC CTA TTT CAT CAA GTT CTA TCC 3'	NLQP
PA-R-1621	5'CTT TTC CCA CTT GTG TGG CTC C3'	NLQP
PA-R-2193	5'ATA GTA GCA TTG CCA CAA CTA 3'	NLQP
HA Primers		
Bm-NS-890R	-ATA TCG TCT CGT ATT AGT AGG AAA CAA GGG TGT TTT	<a href="#">[10,11]</a>
Bm-HA-1-	TAT TCG TCT CAG GGA GCA AAA GCA GGG G	
KH3-	TAC CAA CCG TCT ACC ATK CCYTG	
KH1-	CCTCCAGARTATGCMTAYAAAATTGTC	
4FV2	AGCAGGGGTTCAATCTGTCAAAA	
4RV2	AGTAGAAACAAGGGTGTTTTTAACTA	
NP Primers		

<b>NP-5FV2</b>	5'CA CCG AGT GAC ATC AAC ATC A3'	<b>FLI</b>
<b>NP-F370</b>	5'GAT AAT CAC TCA CCG AGT GAC ATC3'	<b>NLQP</b>
<b>NP-5RV2</b>	5'AAG GGT ATT TTT CTT TAA TTG TCA T A C3'	<b>FLI</b>
<b>NP-R764</b>	5'TCC ATC ATT GCT CTT TGT3'	<b>NLQP</b>
<b>NA Primers</b>		
<b>6FV2</b>	<b>AGCAAAAGCAGGAGTTTAAAATGA</b>	<a href="#">[10,11]</a>
<b>BA-NA-1</b>	<b>TAT TGG TCT CAG GGA GCA AAA GCA GGA GT</b>	
<b>N1- 608-F</b>	<b>AAT AAC AGA CAC CAT CAA GAG TTG</b>	
<b>BaNA-1413-R-</b>	<b>ATA TGG TCT CGT ATT AGT AGA AAC AAG GAG TTT TTT</b>	
<b>N1-R-720 -</b>	<b>CAT GCA CAT TCA GAC TCT TG</b>	
<b>6RV2-</b>	<b>TAGAAACAAGGAGTTTTTTGAACAAAC</b>	
<b>M Primers</b>		
<b>M-7FV2</b>	<b>5'CAAAAGCAGGTAGATGTTGAAAGA 3'</b>	<b>FLI</b>
<b>M-7RV2</b>	<b>5'AACAAGGTAGTTTTTTACTCCAATTC 3'</b>	<b>FLI</b>
<b>M-F710</b>	<b>5'GTTCTTGAAAATTTGCAGG C 3'</b>	<b>NLQP</b>
<b>M-R424</b>	<b>5'GTTGTATGAGACCCATGC 3'</b>	<b>NLQP</b>
<b>NS Primers</b>		
<b>NS-8FV2</b>	<b>5'AAA AGC AGG GTG ACA AAG ACA TAA 3'</b>	<b>FLI</b>
<b>NS-8RV2</b>	<b>5'AGT AGA AAC AAG GGT GTT TTT TAT CA 3'</b>	<b>FLI</b>
<b>NS-R598</b>	<b>5'GAG TTA TCA TTC CAT TCA AGT CC 3'</b>	<b>SEPRL</b>
<b>NS-F327</b>	<b>5'GACTGG TTC ATG CTC ATG CC 3'</b>	<b>SEPRL</b>

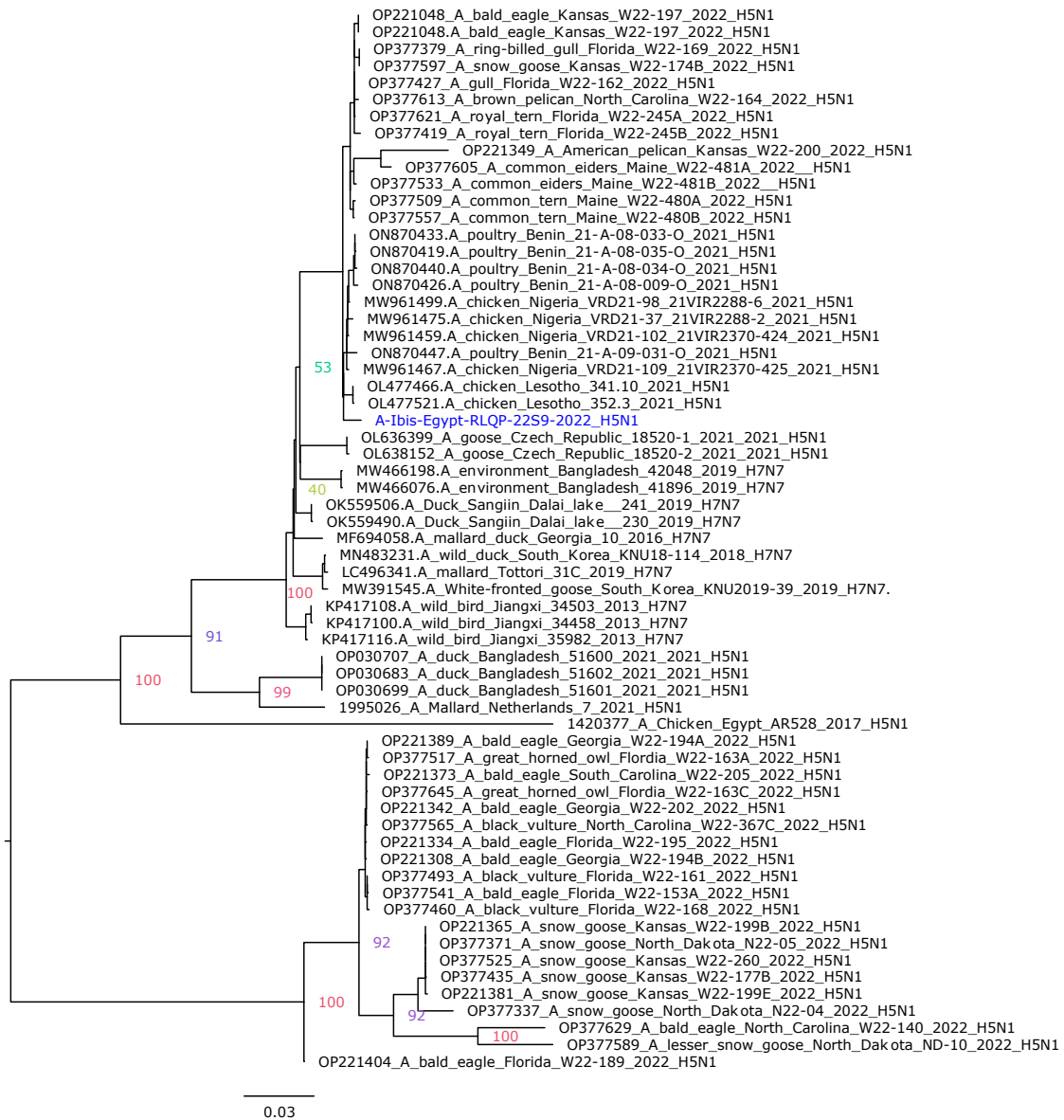
**Table S3.** Epidemiological data and GenBank accession number of detected avian influenza viruses

	Strain name	Collection date	Location	Genbank Accession number
1	A/Greater-Flamingo/Egypt/25/2021	October 2021	Ismailia	OP413044; <u>OP413049</u>
2	A/Common-Blackbird/Egypt/26/2021	November 2021	Damietta	OP413045; <u>OP413050</u>
3	A-Ibis-Egypt-RLQP-229S-2022-H5N1	January 2022	Giza	<u>OP491851; OP491852; OP491854 ;OP491855;</u> <u>OP491860; OP491856; OP491857; OP491859</u>
4	A/Red-back-shrike/Egypt/30/2022	March 2022	Damietta	OP413046; <u>OP413051</u>
5	A/Greater-Flamingo/Egypt/31/2022	March 2022	Damietta	OP413047; <u>OP413052</u>
6	A/Marbled-Duck/Egypt/32/2022	March 2022	Damietta	OP413048; <u>OP413053</u>

**Supplementary Figure S1** Phylogenetic analyses of the PB2(a), PB1(b), PA(c), NP(d), M(e), and NS(f) gene segments.

Phylogenetic trees include representative strains from recent H5N1 subtypes from both Eurasian and North American lineages; in addition to representative H5N1 (clade 2.2.1.2) strains from Egypt and other influenza virus subtypes showing a high nucleotide similarity. The Egyptian HPAI H5N1 strain sequenced in this study is colored in blue. Bootstrap values are shown on main nodes in the phylogenetic tree

**a-PB2**



**b-PB1**



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0.02

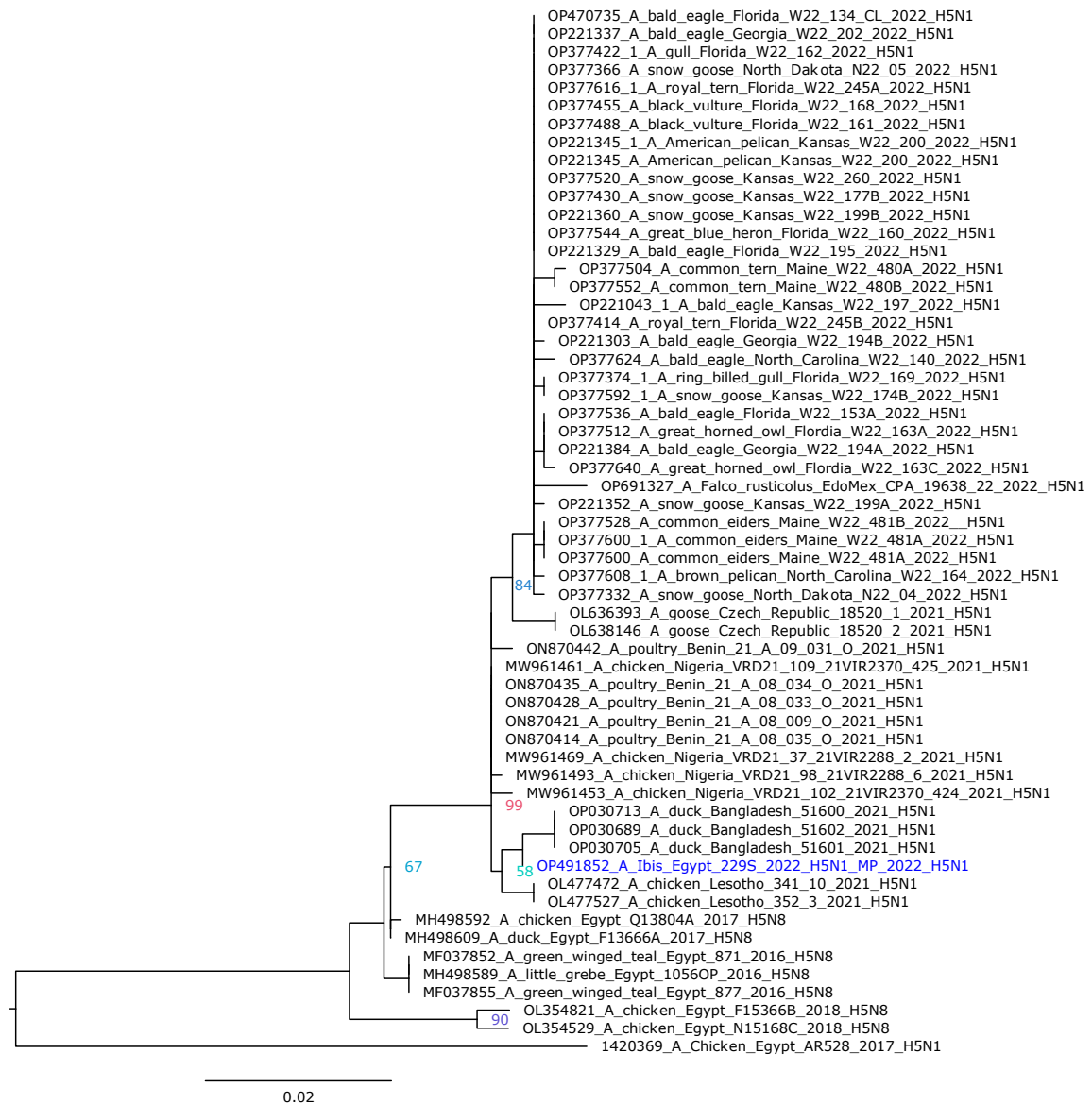
# c-PA

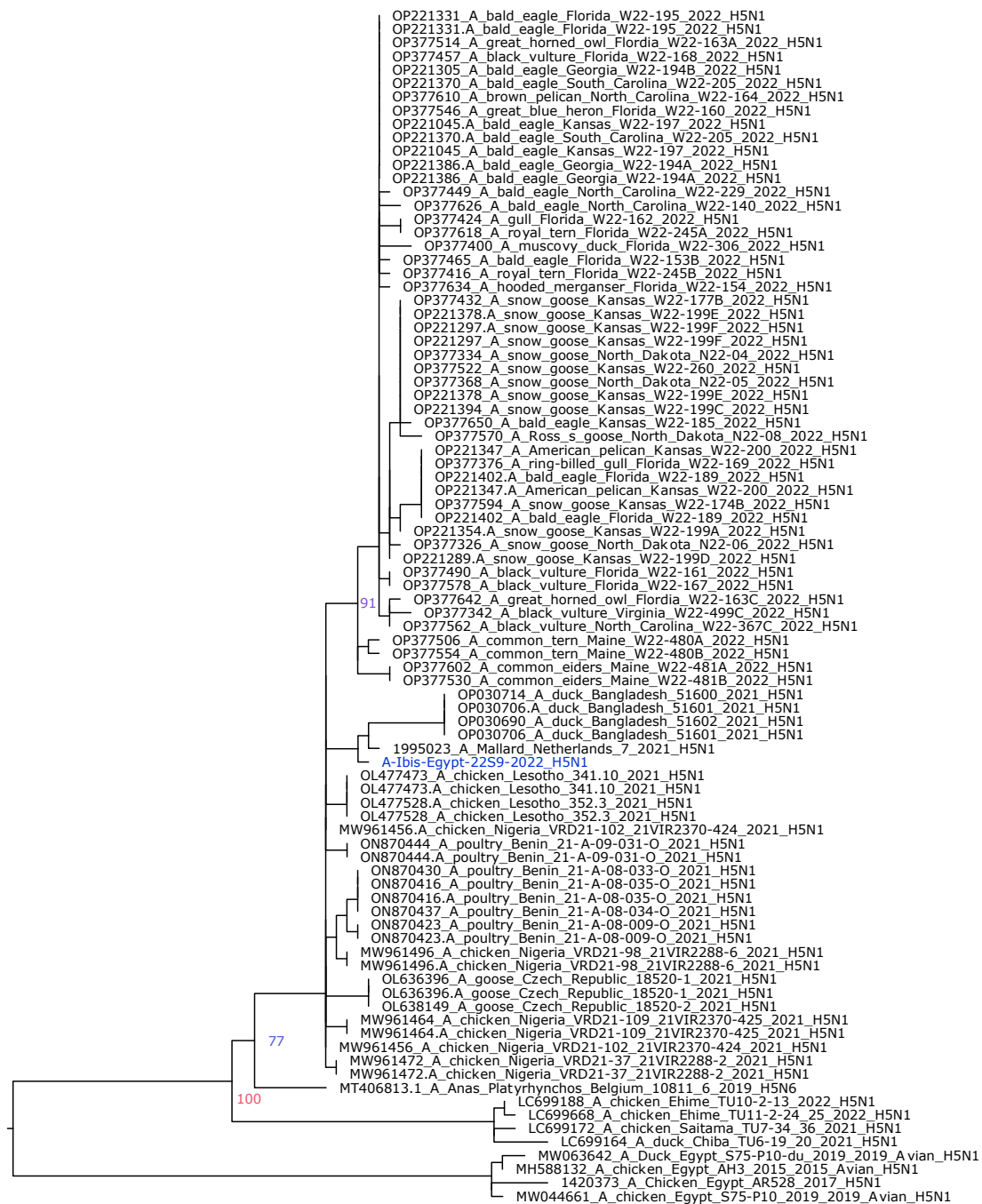


## d-NP



e-M





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