Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009;360:2605-15. DOI: 10.1056/NEJMoa0903810.

(PDF updated May 22, 2009.)

Supplement: Laboratory

Viral RNA was extracted from clarified supernatant fluid using the Qiagen BioRobot M48 workstation with the MagAttract Viral RNA M48 kit (Qiagen, Valencia, CA). For sequence determination, degenerate primers were designed based upon the alignment of all human A (H1N1) sequences available and used in RT-PCR reactions to generate a series of overlapping template amplicons for all genome segments. An M13 sequence tag was added to the 5' end of each primer to be used for later sequencing. Primer sequences are in **Supplementary Table 1.**

RT–PCR was performed using the AccessQuick RT-PCR Kit (Promega, WI). Amplicons were prepared for sequencing by incubating them at 37 °C for 15 min and then at 80 °C for 15 min with ExoSAP-it (USB Corporation, Cleveland, OH) to inactivate remaining dNTPs and primers. Each amplicon was sequenced from each end using M13 primers (F primer: TGTAAAACGACGGCCAGT; R primer: CAGGAAACAGCTATGACC). Sequencing reaction products were resolved on an Applied Biosystems 3730 ABI sequencer. Phylogenetic analyses were performed using the Genetic Algorithm for Rapid Likelihood Inference (GARLI 0.96b7), based on General Time Reversible (GTR) + I + γ4 substitution model.[3] Results are shown in Supplementary figures 1-4.

Adamantane susceptibility was assessed by conventional sequencing and/or pyrosequencing assay[4] using viral RNA extracted from original clinical specimens and/or virus isolates. All samples tested contained the S31N mutation in the M2 protein which has been shown to confer cross-resistance to the adamantane class of anti-influenza drugs.

Nucleotide sequence analysis of both original clinical specimens and virus isolates revealed no predicted amino acid changes in NA previously shown to confer resistance to licensed NA inhibitors in the N1 NA subtype. Susceptibility of virus isolates to the NAIs oseltamivir and zanamivir was functionally assessed in the chemiluminescent neuraminidase inhibition assay using the NAStar™ Kit as previously described.[5] All virus isolates (n=37) exhibited IC_{50} values (concentration needed to inhibit 50% of enzyme activity, nM) characteristic for the oseltamivir- and zanamivir-susceptible influenza viruses. Seasonal influenza A (H1N1) viruses were used as susceptible and resistant controls. The median IC_{50} value for oseltamivir was 0.54 nM , while the median for zanamivir was 0.59 nM.

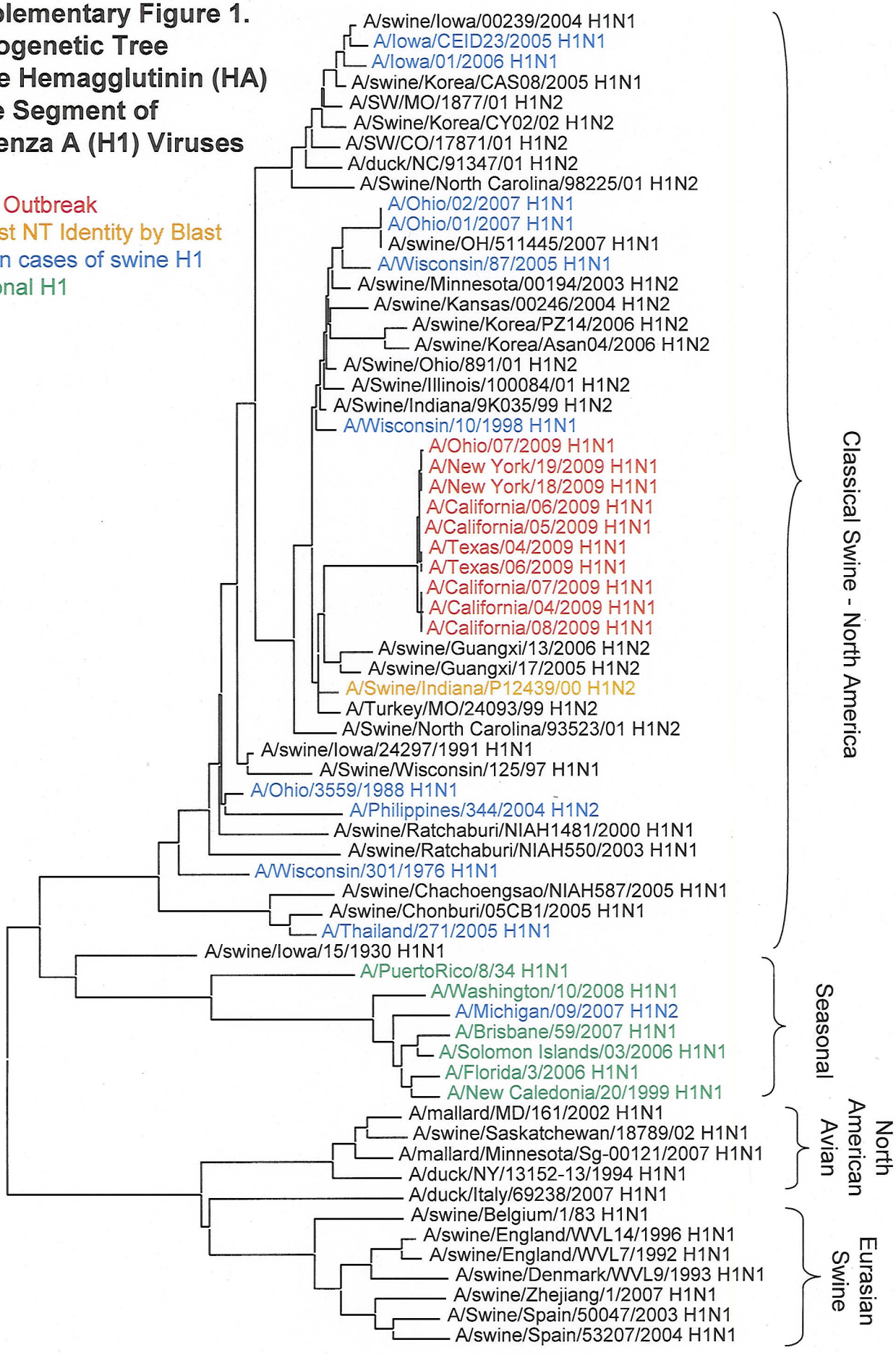
Swine Genome Primer Set

		forward 5'	3'	reverse 5'	3'
PB2					
fragment 1	**1**	tgt aaa acg acg gcc agt agc aaa agc agg tca att		**538**	cag gaa aca gct atg acc ctt cca tra tta cat cyt gtg
fragment 2	**328**	tgt aaa acg acg gcc agt gtr aca tgg tgg aay aga a		**816**	cag gaa aca gct atg acc gct ttg rtc aay atc rtc att
fragment 3	**487**	tgt aaa acg acg gcc agt cct ggt cay gca gac ctc ag		**1019**	cag gaa aca gct atg acc cca aar ctg aag gay gar ctg at
fragment 4	**713**	tgt aaa acg acg gcc agt caa gca gtr trt aca ttg aag t		**1289**	cag gaa aca gct atg acc cct cta act gct ttt ayc atg caa t
fragment 5	**946**	tgt aaa acg acg gcc agt ccr acw gaa gaa caa gct gt		**1509**	cag gaa aca gct atg acc gga gta ttc atc yac acc cat
fragment 6	**1169**	tgt aaa acg acg gcc agt aag caa cca gra gat tgr ttc a		**1740**	cag gaa aca gct atg acc ctg aga cca ytg aat ttt rac a
fragment 7	**1447**	tgt aaa acg acg gcc agt cca agy acm gag atg tca atg aga		**2186**	cag gaa aca gct atg acc ttr ctc art tca ttg atg ct
fragment 8	**1683**	tgt aaa acg acg gcc agt caa tac cta yca rtg gat cat cag aa		**2341**	cag gaa aca gct atg acc tag tag aaa caa ggt cgt t
PB1	**forward**			**reverse**	
fragment 1	**22**	tgt aaa acg acg gcc agt agc aaa agc agg tca att		**477**	cag gaa gct atg acc ctr aaw act tct atr gtg tt
fragment 2	**233**	tgt aaa acg acg gcc agt caa ctc aac ccr att gat ggr cca ct		**843**	cag gaa aca gct atg acc gtt caa gct ttt crc awa tg
fragment 3	**389**	tgt aaa acg acg gcc agt aca agr gtg gac aaa tra c		**1041**	cag gaa aca gct atg acc ctg aac cay tca ggy tga ttt
fragment 4	**711**	tgt aaa acg acg gcc agt tga aca cra tga cca arg a		**1278**	cag gaa aca gct atg acc ttg aac atg ccc atc atc aty cca gg
fragment 5	**974**	tgt aaa acg acg gcc agt aat caa aay cct mga atg tt		**1566**	cag gaa aca gct atg acc agc tcc atg ctr aaa ttr gc
fragment 6	**1139**	tgt aaa acg acg gcc agt caa ata ccy gca gar atg cta gc		**1659**	cag gaa aca gct atg acc cca agr tca ttg ttt atc at
fragment 7	**1489**	tgt aaa acg acg gcc agt atg agy aaa aag aag tcy ta		**1954**	cag gaa aca gct atg acc tca aty tcy tta tgg gtg ac
fragment 8	**1532**	tgt aaa acg acg gcc agt gcy aat tty agc atg gag ct		**2321**	cag gaa aca gct atg acc agt aga aac aag gca ttt
PA	**forward**			**reverse**	
fragment 1	**0**	tgt aaa acg acg gcc agt agc aaa agc agg tac tga t		**493**	cag gaa aca gct atg acc tar tck gcc ttt gtg gcc att tc
fragment 2	**235**	tgt aaa acg gcc agt cca aat gca ctk tta aar cac aga tt		**756**	cag gaa aca gct atg acc tga gaa agc ttg ccc tca atg
fragment 3	**361**	tgt aaa acg acg gcc agt tat gay tac aar gag aa		**989**	cag gaa aca gct atg acc ggt tct ttc cat cca aag aat gtt
fragment 4	**702**	tgt aaa acg acg gcc agt tgc mtt gar aat ttt agr acc ta		**1292**	cag gaa aca gct atg acc tcr cak gcc ttg ttg aac tca tt
fragment 5	**894**	tgt aaa acg acg gcc agt aaa ttr agc att gar gay cca		**1662**	cag gaa aca gct atg acc tcw agt cty ggg tca gtg ag
fragment 6	**1204**	tgt aaa acg acg gcc agt taa gcg att tra agc aat atg a		**2037**	cag gaa aca gct atg acc aay ccy tcy aat tgt ggw gat g
fragment 7	**1444**	tgt aaa acg acg gcc agt aat gca tcc tgt gca gca atg ga		**2057**	cag gaa aca gct atg acc ttg tcc cta aga gcc tga aca a
fragment 8	**1787**	tgt aaa acg acg gcc agt atg aar tgg gga atg gag atg ag		**2233**	cag gaa aca gct atg acc agt aga aac aag gta cct ttt
HA	**forward**			**reverse**	
fragment 1	**1**	tgt aaa acg acg gcc agt ata cga cta gca aaa gca ggg g		**461**	cag gaa aca gct atg acc tca tga ttg ggc cay ga
fragment 2	**351**	tgt aaa acg acg gcc agt acr tgt tac ccw ggr gat ttc a		**943**	cag gaa aca gct atg acc gaa akg gga grc tgg tgt tta
fragment 3	**379**	tgt aaa acg acg gcc agt atg arg arc tra gag agc a		**1204**	cag gaa aca gct atg acc caa tgg crt tyt gtg tgc tc
fragment 4	**736**	tgt aaa acg acg gcc agt agr atg rac tat tac tgg ac		**1340**	cag gaa aca gct atg acc ttc tkc att rta wgt cca aa
fragment 5	**1124**	tgt aaa acg acg gcc agt tgg atg gta ygg tta yca yca		**1541**	cag gaa aca gct atg acc tca taa gty cca ttt ytg a
fragment 6	**1204**	tgt aaa acg acg gcc agt aag atg aay acr car ttc aca g		**1778**	cag gaa aca gct atg acc gtg tca gta gaa aca agg gtg ttt
NP	**forward**			**reverse**	
fragment 1	**1**	tgt aaa acg acg gcc agt cag ggt aga taa tca ctc ac		**553**	cag gaa aca gct atg acc aga gca cat yct ggg atc cat
fragment 2	**296**	tgt aaa acg acg gcc agt atg gtr ctc tct gct ttt gat ga		**757**	cag gaa aca gct atg acc ttt gtg cag ctg ttt gaa att tyc ctt t
fragment 3	**513**	tgt aaa acg acg gcc agt tgg cat tch aat ttr aat gat		**1042**	cag gaa aca gct atg acc ctg rct ctt gtg tgc dgg
fragment 4	**619**	tgt aaa acg acg gcc agt gct gca gtc aar gga rt		**1177**	cag gaa aca gct atg acc aag cra ttt gta cyc ctc tag t
fragment 5	**925**	tgt aaa acg acg gcc agt cct gcy tgt gyg taw gga c		**1565**	cag gaa aca gct atg acc agt aga aac aag ggt att ttt c
NA	**forward**			**reverse**	
fragment 1	**0**	tgt aaa acg acg gcc agt agc aaa agc agg agt		**600**	cag gaa aca gct atg acc ctg gac crg aaa ttc c
fragment 2	**318**	tgt aaa acg acg gcc agt tac aca aaa gac aay agc		**740**	cag gaa aca gct atg acc ggr cca tcg gtc att atg
fragment 3	**536**	tgt aaa acg acg gcc agt ggt cag caa gcg cat gyc atg a		**1063**	cag gaa aca gct atg acc cat aty tgt atg aaa acc
fragment 4	**726**	tgt aaa acg acg gcc agt aat ggr car gcc tcr tac aa		**1346**	cag gaa aca gct atg acc gct gct ycc rct agt cca gat
fragment 5	**941**	tgt aaa acg acg gcc agt tag gat aca tct gca gtg g		**1452**	cag gaa aca gct atg acc agt aga aac aag gag
M	**forward**			**reverse**	
fragment 1	**0**	tgt aaa acg acg gcc agt agc aaa agc agg tag		**473**	cag gaa aca gct atg acc gca atc tgy tca cak gt
fragment 2	**223**	tgt aaa acg acg gcc agt cac cgt gcc cag tga gcg		**750**	cag gaa aca gct atg acc tca ytt gaa ycg ytg cat
fragment 3	**383**	tgt aaa acg acg gcc agt tct gct ggw gca ctt gcc agt tg		**1027**	cag gaa aca gct atg acc agt agm aac aag gta gt
NS	**forward**			**reverse**	
fragment 1	**24**	tgt aaa acg acg gcc agt agc aaa agc agg gtg aca aag aca		**482**	cag gaa aca gct atg acc tcg gtg aaa gcc ctt a
fragment 2	**250**	tgt aaa acg acg gcc agt tga ggc wyt taa aat gac ca		**890**	cag gaa aca gct atg acc agt aga aac aag ggt gtt ttt tat
fragment 3	**418**	tgt aaa acg acg gcc agt aaa gcd aay ttc agt gtg		**742**	cag gaa aca gct atg acc ttc aat hag cca tct ta

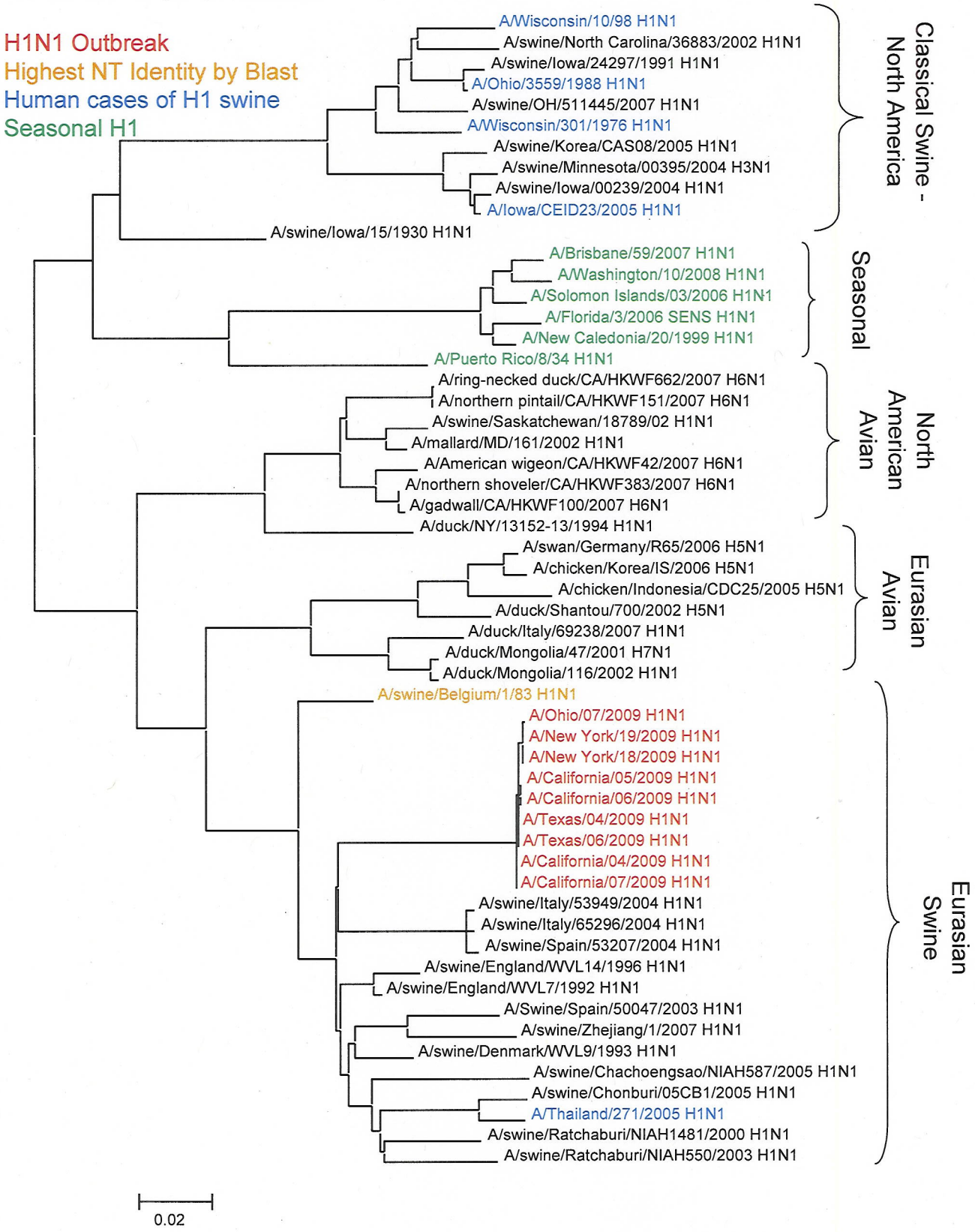
m13 forward	**m13 forward**	tgt aaa acg acg gcc agt
m13 reverse	**m13 reverse**	cag gaa aca gct atg acc

Supplementary Figure 1. Phylogenetic Tree of the Hemagglutinin (HA) Gene Segment of Influenza A (H1) Viruses

H1N1 Outbreak
Highest NT Identity by Blast
Human cases of swine H1
Seasonal H1

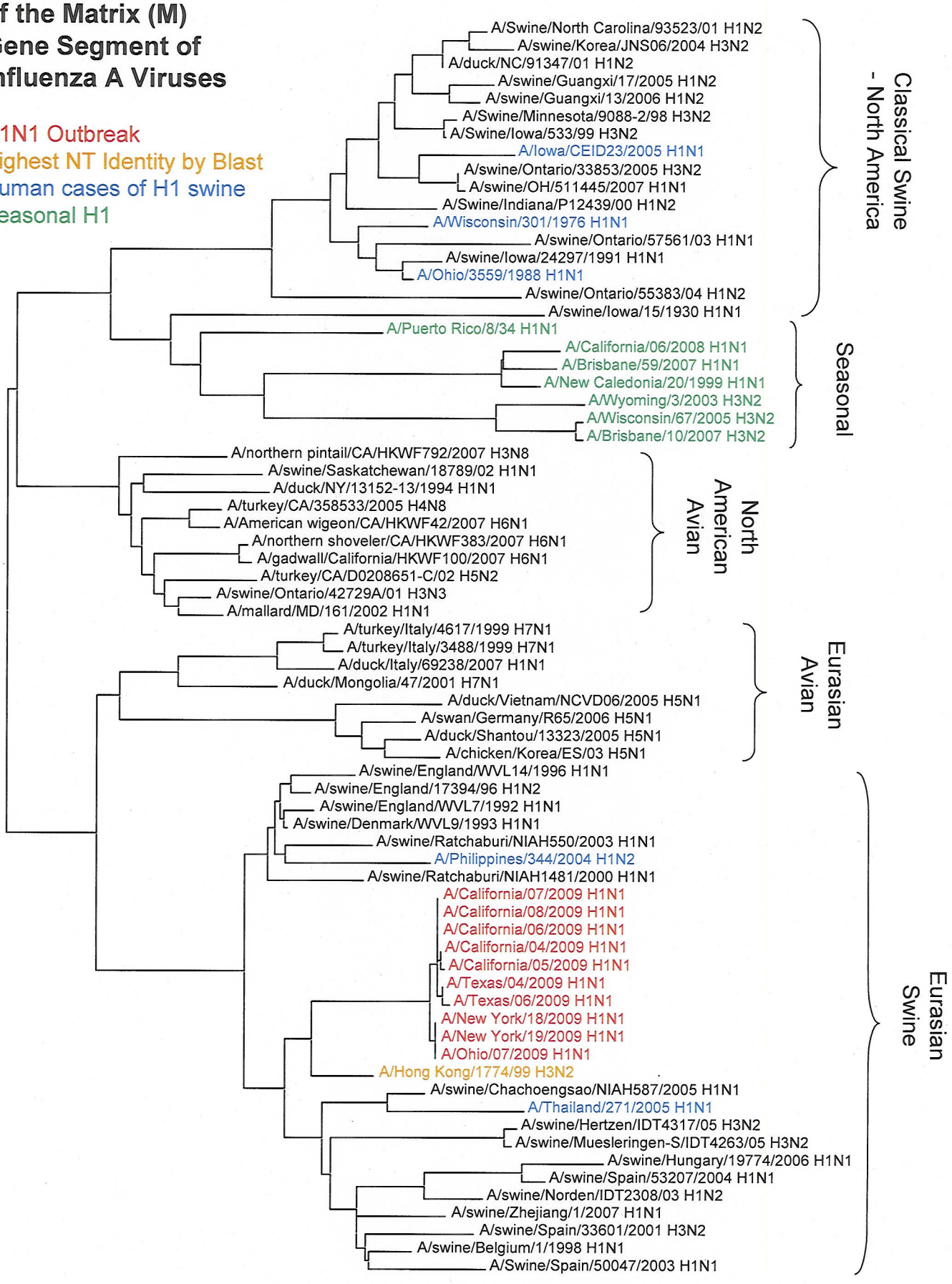


Supplementary Figure 2.
Phylogenetic Tree of the Neuraminidase (NA) Gene Segment of Influenza A (N1) Viruses



Supplementary Figure 3.
Phylogenetic Tree of the Matrix (M) Gene Segment of Influenza A Viruses

H1N1 Outbreak
Highest NT Identity by Blast
Human cases of H1 swine
Seasonal H1



Accession Numbers For Strains Included In Supplemental Figure 2:

	HA	NA	M
A/CALIFORNIA/04/2009	FJ966082	FJ966084	FJ966085
A/CALIFORNIA/05/2009	FJ966952	FJ966956	FJ966954
A/CALIFORNIA/06/2009	FJ966960	FJ971075	FJ966962
A/CALIFORNIA/07/2009	FJ969540	FJ984386	FJ969537
A/CALIFORNIA/08/2009	FJ971076		FJ969532
A/NEW YORK/18/2009	FJ984355	FJ984350	FJ984348
A/NEW YORK/19/2009	FJ984394	FJ984390	FJ984388
A/OHIO/07/2009	FJ984397	FJ969520	FJ984395
A/TEXAS/04/2009	FJ981612	FJ966981	FJ966980
A/TEXAS/06/2009	FJ984385	FJ984383	FJ984381