

Summary of laboratory sequence data for swine influenza viruses from two human cases in CA

A/California/04/2009 Case A - 10 y.o. boy, San Diego, CA

A/California/05/2009 Case B – 9 y.o. girl, Brawley, CA

Full or partial sequences are complete for each of the 8 genes for isolates and/or clinical material from both cases. Sequence data indicate that viruses from both cases are swine influenza viruses with hemagglutinin (HA) genes of the classical swine H1N1 subtype. Influenza viruses that infected both Case A and B are very similar to each other with respect to sequence data for all 8 gene segments. Only 1 to 5 nucleotide differences (encoding 1 to 3 amino acids) have been detected between the available sequences for each corresponding gene for viruses that infected these two cases, except for the M genes which are identical at the nucleotide level.

Nucleotide Blast results for the virus A/California/04/2009 isolated from Case A (Table 1), show that the HA, PB2, PB1, PA, NP, NS genes contain gene segments from influenza viruses isolated from swine in North America, while the NA and M genes are most closely related to corresponding genes from influenza viruses isolated in swine population in Eurasia. This particular combination of swine influenza virus segments has not been reported in the literature for swine influenza viruses isolated in the United States.

The HA gene of A/California/04/2009 is related to classical swine H1 influenza viruses similar to A/swine/Indiana/P12439/00 H1N1 circulating in the U.S. and Asia (Figure 1). Although the HA belongs to the same lineage as recent human cases of H1N1 classical swine triple reassortants, HAs of related viruses differ from that of the A/California/04/2009 virus by approximately 20-30 aa in the HA1 regions alone.

The neuraminidase (NA) gene of A/California/04/2009 has closest nucleotide homology to the Eurasian lineage of swine influenza viruses, including viruses such as A/swine/Belgium/1/83 H1N1 (Figure 2). This is in contrast to the recent human cases of H1N1 classical swine triple reassortants which contain NA genes from the North American swine lineage. The Eurasian and North American NA lineages are highly divergent, with over 77 amino acids differences in the NA between these lineages. In preliminary tests the CA viruses appear to be susceptible to the neuraminidase inhibitors (oseltamivir and zanamivir); however additional antiviral testing susceptibility is ongoing.

Similar to the NA, the M gene of A/California/04/2009 has closest homology to M genes of the Eurasian lineage of swine influenza viruses (Figure 3). Analysis of the M2 protein sequence from the two CA viruses shows a Ser-31-Asn mutation conferring resistance to M2 blockers (amantadine and rimantadine). This

phenotype is typical for recent Eurasian lineage swine influenza viruses, but has not been previously seen in American swine influenza viruses.

Sequences of the PB1, PB2, PA, NP (replication complex) and NS genes of both CA viruses have the closest homology to swine influenza viruses isolated in the U.S. from the North American swine lineage.

The completed HA, NA and M gene sequences for A/California/04/2009 are available in the publically accessible GISAID sequence database and have the following accession numbers:

HA: SequenceID: EPI176470

SequenceName: 2009712049_seg4

Length: 1701

Isolate: A/California/04/2009

NA: SequenceID: EPI176472

SequenceName: 2009712049_seg6

Length: 1410

Isolate: A/California/04/2009

M: SequenceID: EPI176471

SequenceName: 2009712049_seg7

Length: 972

Isolate: A/California/04/2009

Table 1. NCBI Blast Results A/California/04/2009 2009712049 IR							
Gene	Length NT	NCBI	Strain	Lineage	Subtype	Identities	Additional Information
HA	1701	AF455600.1	A/Swine/Indiana/P12439/00	North American Swine	H1N2	1621/1701 (95%)	
NA	1410	AJ412690.1	A/Swine/Belgium/1/83	Eurasian Swine	H1N1	1302/1410 (92%)	
M	972	AJ293925.1	A/Hong Kong/1774/99	Eurasian Swine	H3N2	945/972 (97%)	Human case of H3N2 Eurasian Swine
PB2	2264	EU301177.2	A/swine/Korea/JNS06/2004	North American Swine	H3N2	2186/2264 (96%)	
PB1	2274	AF342823.1	A/Wisconsin/10/98	North American Swine	H1N1	2203/2274 (96%)	
PA	925	AF455717.1	A/Swine/North Carolina/93523/01	North American Swine	H1N2	877/925 (94%)	
NP	1497	AF251415.2	A/Swine/Iowa/533/99	North American Swine	H3N2	1449/1497 (96%)	
NS	838	AF153262.1	A/Swine/Minnesota/9088-2/98	North American Swine	H3N2	809/838 (96%)	