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**Molecular characterization of influenza a viruses from swine for potential virulence markers**

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**Introduction**: As influenza A viruses continue to emerge and evolve through reassortment and antigenic drift in the US swine population, novel strains have arisen over the last decade that have resulted in changes in influenza epidemiology and persistence within endemically infected populations of pigs. The hemagglutinin (HA) gene of all influenza viruses that were detected in samples submitted from one endemically infected farm system (farm M) have been sequenced since 2005. In 2008, a two amino acid (AA) insertion first appear at position 156 using the H1 numbering system and this unique insertion has been repeatedly detected in this farm system over the last two years. The viruses with the insertion were isolated from pigs in specific barns in a multi-site rearing system from farm M that were experiencing increases in piglet mortality. To better understand how this insertion relates to the pathogenicity of these influenza viruses, five viruses with the insertion and three potential ancestral viruses without the insertion were selected for further molecular analysis.

**Materials and Methods**: The full genomes of eight viruses were sequenced at St. Jude Children's Research Hospital in Memphis, TN. The sequences were analyzed using MEGA 4 with Clustal W alignment the three-dimensional models of the antigenic sites were examined using PyMOL.

**Results:** Phylogenetic analysis of the viruses revealed that the isolates clustered tightly together with the highly pathogenic virus A/Sw/OH/51145/2007 (H1N1) in the SwH1 gamma clade (Figure 1). Analysis of the protein sequence revealed that the two AA insertion at position 156 disrupted the Sb and Sa antigenic sites which are proximal to the HA receptor binding pocket in the three-dimensional protein structure. This was the only variation in the antigenic sites for two of the five viruses. One virus also had one AA change at position 70. Two other viruses also had AA changes at 74, 139, 162 and 168.

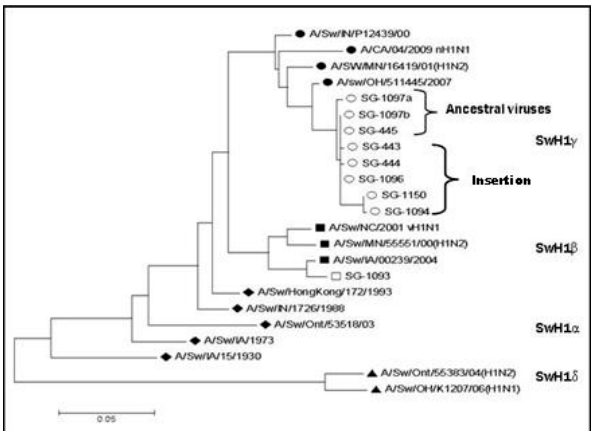


**Figure 1.** Phylogenetic tree of eight viruses (O) analyzed from farm M.

| Antigenic site | SG-443 and SG-1096 | | SG-444 | | SG-1094 and SG-1150 | |
|---|---|---|---|---|---|---|
| (Total no. AA residues) | No. changes | AA position | No. changes | AA position | No. changes | AA position |
| Sa (13) | 0 | n.a. | 0 | n.a. | 1 | 162 |
| Sb (9) | 1 | 156 | 1 | 156 | 1 | 156 |
| Ca (14) | 0 | n.a. | 0 | n.a. | 2 | 139,168 |
| Cb (8) | 0 | n.a. | 1 | 70 | 1 | 74 |

**Table 1**. The number and position of AA changes in antigenic sites of HA1 of the viruses containing the insertion compared to the ancestral viruses.

**Discussion:** Molecular characterization of influenza viruses has become one of the key tools used by researchers to examine the continual changes that are found in viruses isolated from the U.S. swine population. By sequencing the viruses that are endemic in pigs, we can screen the circulating viruses for significant genetic changes, compare multiple viruses isolated from the same population over time, as well as to viruses in available vaccines. These changes will provide a benchmark for evaluating how the virus evolves in swine. This information is critical for understanding the epidemiology of the disease caused by the virus within a farm or production system, especially when the molecular data can be combined with clinical signs and post-mortem findings.