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POLYMERASE COMPLEX ACTIVITY OF SWINE H1 INFLUENZA A VIRUS WITH DIFFERENT GENOME PATTERNS AND PHENOTYPES

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Background and Objectives

Influenza A viruses (IAV) have considerable genetic diversity. We assessed the pathogenesis and transmission of five representative swine lineage H1 viruses in pigs. Viruses were assigned a genome constellation using HA and NA clade, with internal gene lineage (TRIG=T or Pandemic=P) in the order of PB2, PB1, PA, NP, M, and NS. The 5 viruses replicated in the nose and lungs, but 1A.3.3.3/N1c-TTPPPT presented significantly higher lung lesions while 1A.1.1.3/N2-02-TTPTPT had higher nasal shedding. We evaluated whether the polymerase complex activity (PCA) of the different genomes influenced in vivo phenotypes.

Material and Methods

PCA using a pHW-SP reporter plasmid containing Gaussia luciferase (GLuc) in a minigenome assay was assessed. The PB2, PB1, PA, and NP segments of the five viruses (1A.1.1.3/N2-02-TTPTPT, 1B.2.2.1/N2-02-TTTTPT, 1B.2.2.2/N2-02-TTTPPT, 1B.2.1/N2-02-TTTTPT, and 1A.3.3.3/N1c-TTPPPT) were cloned into the pHW-SP reporter plasmid (gift of D.Perez, UGA, USA), transfected into MDCK-SIAT1 cells, and incubated at 37°C and 39°C. Cell supernatant were collected at 12, 24, 48, and 72 hours post-transfection (hpt). Reporter gene expression with GLuc activity was used to measure PCA.

Results

At 37°C, 1A.1.1.3/N2-02-TTPTPT had significantly higher PCA at 24 and 72hpt than others, consistent with the early peak of virus replication in the nose. By 48hpt, all five minigenomes had PCA at 37°C and remained active until 72hpt. At 39°C, the PCA of 1A.1.1.3/N2-02-TTPTPT was significantly higher at 48hpt than others. 1A.3.3.3/N1c-TTPPPT did not show PCA that was significantly different from others at any timepoint at 39°C.

Discussion and Conclusion

1A.1.1.3/N2-02-TTPTPT demonstrated PCA at both temperatures that matched the high viral replication in the nose and lungs. The 1A.3.3.3/N1c-TTPPPT showed lower PCA at both temperatures. Host factors and specific viral gene combinations, like HA and NA pairing with an efficient polymerase complex, are likely involved in increased replication of swine adapted viruses rather than PCA alone. These data demonstrate that diverse internal IAV genes affect phenotype, and specific combinations may have an advantage due to PCA in the swine host.