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Polybasic cleavage site is essential for replication competent neuraminidase-negative HPAIV H5N1

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Question: The role of the hemagglutinin (HA) cleavage site of a replication competent but avirulent neuraminidase-negative highly pathogenic avian influenza virus (HPAIV) H5N1 should be investigated.
Methods: Virus recovery, growth kinetics and plaque sizes were measured. Virulence was studied by experimental infection of chicken and ferrets. Reverse genetics were used to generate defined virus constructs.
Results: Characterization of the NA-negative HPAIV revealed a loss of enzymatic activity. Reduced viral titers were observed until 48h post infection (p.i.) in the cell culture supernatant, while titers detected 72 h p.i. were similar to titers of the wild-type H5N1 virus. Plaque sizes of the NA-negative HPAIV were severely reduced by about 90% and application to chicken and ferrets characterized the virus variant as avirulent despite the presence of a polybasic HA-cleavage site. A recombinant H5-virus with the non-functional NA-segment could be generated, however, presence of the polybasic HA-cleavage site was a prerequisite for virus progeny. Attempts to generate the same recombinant virus with a monobasic cleavage site failed, while deletion of only 2 basic amino acids was tolerated.
Conclusions: Characterization of the NA-negative HPAIV H5N1 indicated a prominent impact of the neuraminidase on 'cell-to-cell-spread' that may count for the growth delay. However, monobasic HA of subtype H5 could not compensate for the neuraminidase negative phenotype, while neuraminidase negative virus - exhibiting a polybasic HA - was replication competent without any supplementation. Overall, the variant reported may facilitate further studies addressing the neuraminidase and its role in virus replication and pathogenicity.

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