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## **Genetic and antigenic characterization of influenza A virus circulating in Danish swine during the past decade.**

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Influenza A virus has been endemic in Danish swine for the last 30 years, with H1N1 and H1N2 being the dominating subtypes. The purpose of this study was to investigate the genetic and antigenic evolution of the influenza viruses found in Danish swine during the last 10 years. A total of 78 samples were isolated in MDCK cells, RNA extracted and the hemagglutinin and neuraminidase genes full length sequenced. In addition, the isolates were tested in hemagglutination inhibition (HI) tests against a panel of known antisera raised against a range of European swine influenza virus isolates. Phylogenetic analysis of the HA and NA genes revealed continuous evolutionary drift as expected for RNA viruses with low mutational selection pressure. Estimated selection pressures indicated that more purifying and less diversifying selection controlled the H1 evolution. The mean rates of synonymous and non-synonymous substitutions for H1, N1 and N2 were found to be in agreement with previously observed values for Eurasian swine lineages. Calculation of possible glycosylation sites in the hemagglutinin gene revealed that the H1N2 and H1N1 subtypes had three well conserved glycosylation sites in common. The results of the HI tests were analysed by antigenic cartography to quantify the antigenic relationship between the virus isolates. The antigenic cartography map showed that most of the Danish viruses were antigenic very similar, with only a few outliers. In conclusion, this study provided an important contribution to the complex epidemiology of circulating swine influenza virus in Denmark and indicates that vaccine development targeted against Danish H1N1 and H1N2 need only to include few components for the induction of cross protection against the predominant strains.

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