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Surveillance programs in Denmark has revealed the circulation of novel reassortant influenza A viruses in swine

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Introduction

Swine influenza is a respiratory disease caused by multiple subtypes of influenza A virus. Swine influenza virus (SIV) is enzootic in swine populations in Europe, Asia, North and South America. The influenza A virus genome consist of eight distinct gene segments and SIV subtypes are defined by the combination of the gene segments hemagglutinin (HA) and neuraminidase (NA).
In most European countries, the avian-like (av)H1N1, the 2009 pandemic variant (H1N1pdm09), H1N2 and H3N2 subtypes have constituted the dominating SIV subtypes during recent years. In Denmark, the H1N2 subtype is a reassortant between avH1N1 and H3N2 which is different from the dominating European H1N2 subtype (*1*). The prevalence of the H1N1pdm09 virus in swine has increased since 2009 in some countries including Denmark. Here we present the results of the national passive surveillance program on influenza in swine performed from 2009-13.

Materials and Methods

Clinical samples submitted for diagnostic purposes from Danish pigs with respiratory diseases since 2009 were tested for SIV and included in the survey. Between 280 and 527 submissions were tested per year. Routinely, samples were initially tested by pan-influenza A real-time reverse transcriptase PCR assays (rRT-PCR) targeting the M or the NP gene (*1*). All positive samples were then tested by an in-house real-time PCR assay specific for the H1N1pdm09 virus HA gene. Since 2011, selected SIV positive samples were subtyped by partial sequence analysis of the HA and NA genes and a subset of those were full genome sequenced by next generation sequencing. The sequences were analysed and compared to virus sequences present in the GISAID database using standard bioinformatics tools (*1, 2*).

Result

The percentage of submissions that tested positive for SIV increase over the years reaching 47% in 2013. The subtype was determined by phylogenetic analyses of the partial HA and NA gene sequences which showed that the HA belonged to two different H1 lineages - the H1 avian-like SIV and the H1 pandemic (H1pdm09). The NA genes belonged to two different N1 lineages - the N1 avian-like SIV and N1 pandemic (N1pdm09). The majority of samples harboring an N2 gene belonged to the H3N2 SIV lineage, however, a few samples harbored an N2 gene most closely related to human H3N2 viruses from the mid-1990s (*2*). When combining the HA and NA phylogenetic data the results revealed that the two most common subtypes in Danish swine were H1N1 and H1N2 with avian like HA genes. The third most common subtype was the pandemic H1N1pdm09 virus. A single H3N2 different from the vH3N2 virus *(5)* was found in 2013. The results further revealed the presence of new reassortant SIV subtypes in the European swine population comprising either a new combination of known circulating SIV genes or the presence of an N2 gene not previously detected in pigs (*2*). In total, new reassortant SIV subtypes comprised 10 % of the characterized SIV viruses.

Conclusions and Discussion

The results of the passive surveillance program in Denmark revealed that almost 10 % of the circulating viruses were reassorted viruses which were different from the known circulating viruses in one or more gene segments. There is a significant movement of living pigs within Europe which makes it likely that new reassortants will spread. Indeed, reassortants identical to the viruses found in Denmark have recently been detected in Germany (*3*). This further emphasize that European networks on exchange of SIV data like the successful ESNIP3 project should be supported also prospectively.
Detailed knowledge on the genetic and antigenic characteristics of circulating influenza A viruses in swine is of veterinary importance in respect to choice of vaccines. Thus, the use of vaccines with heterologous HA vaccine antigens may elicit insufficient protection or even potentiate disease following natural infection (*4*). In addition, these findings is also important in a human health perspective because new re-assortments may generate new viruses with increased zoonotic potential as seen for the H3N2v virus in the US (*5*).

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