MOLECULAR EPIDEMIOLOGY OF SWINE INFLUENZA VIRUS IN FRANCE: IDENTIFICATION OF NOVEL H1N1 REASSORTANTS

N. Franck[1], S. Queguiner[1], S. Gorin[1], E. Eveno[2], C. Fablet[2], F. Madec[2], G. Kuntz-Simon[1]

[1]AFSSA-LERAPP, Swine Virology and Immunology Unit, Ploufragan, France;
[2]AFSSA-LERAPP, Pig Epidemiology and Welfare Unit, Ploufragan, France

Key words: *swine influenza, epidemiology, reassortant*

Introduction and Objectives

Swine influenza is a highly contagious acute viral disease of the respiratory tract in pigs. Three major subtypes of influenza A virus, H1N1, H3N2 and H1N2, co-evolve in pigs, but various lineages of each one can be distinguished depending on the world area. The majority of swine influenza A H1N1 viruses circulating in Europe are of avian origin. H3N2 SIV circulating since the mid 1980s in European pigs are reassortants between the initial H3N2 strain of human origin and an avian-like H1N1 strain, from which they inherited the internal genes. H1N2 viruses arose by genetic reassortment of H1N1 and H3N2 strains and currently circulating strains possess a haemagglutinin (HA) closely related to those of human H1N1 viruses circulating in the early 1980s. Since 2002, no report has been done to characterize on a molecular level, circulating SIV strains in France (Marozin et al., 2002). In this study we examined the molecular characteristics of HA1 gene of swine influenza viruses isolated from 2000 to 2006 in pig farms in Brittany (France). Our first investigations of the genetic relationships between SIV strains defined the existence of two novel reassortants.

Material and methods

Viruses. These were isolated from nasal swabs during outbreaks of respiratory disease in pigs on farms in the Brittany area, by passage in the amniotic and allantoic cavities of 10-day-old fertile hen eggs or in cultures of MDCK cells.

Multiplex RT-PCR assays. They were performed as described by Kuntz-Simon et al. (2005). Briefly, extracted RNA was reverse transcribed with random primers and cDNA used in two separate multiplex reactions. Each one was achieved using two sets of primers, designed to identify either H1 and H3 regions or N1 and N2, respecitvely. Amplicons were analysed by 1,5% agarose gel electrophoresis.

Gene sequencing and analyses.

Viral RNA was prepared from samples of infected cell culture fluid or allantoic fluid by Trizol extraction and ethanol precipitation. Extracted RNA was reverse transcribed using Vgen primer (5'AGCAAAAGCAGG3') with MMLV reverse transcriptase (Promega). HA1 PCR was done with subtype specific primers proposed by the ESNIP1 program. The resultant amplified products of predicted sizes were purified with a QIAquick gel extraction kit (Qiagen). PCR fragments were sequenced using the ABI Prism dye terminator cycle 3.1 sequencing kit with subtype specific HA1 primers and run on an ABI 3130 genetic analyser.

Partial nucleotide sequence data were assembled using Contig Express, multiple sequence alignments done using Align X (VectorNTI Advance 9, Invitrogen). Nucleotide sequences were traduced using Six Frame Translation Software. Phylogenetic analyses were done at the nucleotide and protein level using the neighbour-joining algorithm with correction for multiple substitutions using 1000 bootstrap trials. TREEVIEW was used to produce the phylogenetic tree diagram.

Results

Our laboratory actually has a collection of about eighty SIV isolates collected during the period of 2000 to 2006. Strain subtype was determined by multiplex RT-PCR assays. It appeared that two subtypes of SIV, H1N1 and H1N2, are currently circulating in Brittany (France). The prevalence of each subtype is about 50%. No H3N2 strain could be isolated in our study. We further focused on genetic comparisons of partial HA1 regions, between H1N1 and H1N2 strains at both, nucleotide and protein level.

H1N1 viruses: Seven-hundred-and-eight nucleotides of HA nucleotide sequence (corresponding to positions 1-322 and 616-1002 of the open reading frame) were sequenced from fifteen virus strains isolated during the years 2005-2006 and two strains from 2001 and used for phylogenetic analyses. With one exception, all the virus strains isolated during the years 2005-2006 are closely related to the HA1 of A/Sw/IleVilaine/1455/99, an antigenic variant of the avian-like H1N1 swine isolate Sw/Finistì re/2899/82. One strain isolated in 2006 (Sw/Ploufragan/0190/06) and the two strains isolated in 2001(Sw/Ploufragan/60293/01 and Sw/Ploufragan/98574/01) showed a HA more closely related to the HA of human H1N1 viruses A/Brazil/11/78 and A/England/333/80 corresponding to the HAs actually found in circulating H1N2 strains.

H1N2 viruses: Six-hundred-and-fifty nucleotides of HA nucleotide sequence (coresponding to positions 1-322 and 616-944 of the open reading frame) were analysed from 10 isolates (isolated from 2000-2006). Phylogenetic analysis showed a clear division of the H1N2 viruses into two major HA groups: the first group contained 3 isolates (from 2001-2006) closer related to older H1N2 strains like Sw/Scotland/410440/94; the second group contained 7 isolates (from 2000-2006) with a HA more closely related to the HA of Sw/Cotes d'Armor/800/00.

Phylogenetic trees of partial HA1 nucleotide and protein sequences grouped H1N1 strains Sw/Ploufragan/0190/06, Sw/Ploufragan /60293/01 and Sw/Ploufragan/98574/01, within the H1N2 lineage.

Discussion and Conclusions

Genetic comparisons done at nucleotide and protein levels showed that there was a relatively low homology between the HA1 of H1N1 and H1N2 viruses. With three exceptions, the HAs of the swine H1N1 viruses were of avian origin and closely related to Sw/IV/1455/99, the antigenic variant of the earlier circulating Sw/Finistì re/2899/82 strain. Thus our analysis confirmed the results previously obtained by Marozin et al. (2005), and clearly show that this variant who has emerged among H1N1 viruses, circulating in pigs since the early 1980s, is now predominant.

Phylogenetic analysis of partial HA1 sequence of H1N2 strains showed much more divergence within this subytype. Our results clearly pointed out the existence of two phylogenetically distinguishable HA groups, with the majority of the isolates more closely related to Sw/CA/800/00, generated by a reassortment between a H1N2 strain and Sw/IV/1455/99 (H1N1)-like viruses. Moreover, the phylogenetic tree showed a marked heterogeneity among the H1N2 strains, which contrasts with the high similarity among H1N1 circulating viruses. Concerning the H1N1 isolates with a HA1 gene phylogenetically close to HA1 of H1N2 isolates, Sw/Ploufragan/0190/06 was closer to Sw/CA/800/00, whereas HA1 genes of Sw/Ploufragan/60293/01 and Sw/Ploufragan/98574/01 were more closely related to Sw/Scotland/410440/94. Thus we hypothesize that the origin of these 3 reassortants is different: the 2001 isolates could be reassortants between an avian-like H1N1 strain and a H1N2 strain closely related to Sw/Scotland/410440/94, whereas the more recent strain could be a reassortant between an avian-like H1N1 strain and a H1N2 strain more closely related to Sw/CA/800/00. Sequencing of internal genes and NA of these 3 strains is under progress, in order to determine precisely the origin of the viruses genes.

Acknowledgements

We thank Dr Buffereau JP and Baudouard MA, LDA22, Ploufragan, for providing some virus strains. This research was supported by the "Region Bretagne" Fluporc Program. AFSSA-LERAPP is partner of the European Coordination Action ESNIP2 (SSPE-CT-2005-022749) *(European Surveillance Network for Influenza in Pigs)*.

References

1. Kuntz-Simon G. et al. (2005) Evaluation of multiplex RT-PCR assays for detection and subtyping of swine influenza H1N1, H3N2 and H1N2 viruses in French Clinical Samples. Proccedings of the IABS International Congress, p.97. Saint Malo, France.

2. Marozin S. et al. (2002) Antigenic and genetic diversity among swine influenza A H1N1 and H1N2 viruses in Europe. J Gen Virol, 83, 735-745.