Influenza A(H3) whole genome analysis: searching causes for vaccine failure in 2011/2012

Pedro Pechirra¹, Ana Carina Maia¹, Daniel Ataíde Sampaio², Vítor Borges³, João Paulo Gomes³, Raquel Guiomar¹





- EE149

— MS43 — EE27

U111

— SU15

Hemagglutinin

gene

¹ Portuguese Influenza Reference Laboratory, Infectious Diseases Department; ² Innovation and Technology Unit, Human Genetic Department; ³ Bioinformatics Unit, Infectious Diseases Department; National Institute of Health Dr Ricardo Jorge,

ectious Diseases Department; National Institute of Health Dr Ricardo Jorge,
Lisbon, Portugal

Background:

The 2011/2012 season in Portugal was characterized by an excess mortality and influenza vaccine failures. Predominant influenza A(H3) viruses with new antigenic properties were associated with potential host immune evasion. The aim of this study was to determine possible viral genetic causes that may be associated with cases of vaccine failure by performing a whole genome-based comparison of viruses detected in vaccinated (vacc) and unvaccinated (unvacc) individuals in 2011/2012 season.

Methods:

In 2011/12 season, 678 nasopharyngeal swabs from ILI cases were analyzed by the Portuguese NIC. Were detected 260 influenza A(H3) and 6 B/Yamagata viruses. For whole genome sequencing (WGS) 25 A(H3) positive samples, 20 from vacc and 5 from unvacc individuals were selected. Each of the influenza genomic segments was submitted to standard or multiplex PCR amplification, and further sequenced on a MiSeq platform. Reads mapping was subsequently performed using Bowtie2, followed by SNP/Indel calling using SAMtools/BCFtools. Variant nucleotide sites were carefully confirmed through visual inspection using the Integrative Genomics Viewer. Multiple alignments, phylogenetic and mutational analysis were performed using MEGA software 6.0.

Results:

- Influenza A(H3) viruses clustered into different genetic clades, reflecting the clades circulating in Portugal in 2011/2012.
- Twenty viruses belonged to the genetic clade 6 (reference strain A/lowa/19/2010) and 5 viruses have clustered in the genetic clade 3 (Figure 1 and Tables Ia and Ib).
- Viral genomes were highly similar at nucleotide level, ranging 98.2% 100.0% of similarity.
- Matrix and nucleoprotein genomic segments were the most conserved (Table Ib),
- Highest number of substitutions leading to amino acid changes was observed in hemagglutinin and neuraminidase (Table Ia).
- The deduced amino acid sequences of viral proteins did not reveal any particular feature assigned to the group of vacc or unvacc individuals (Tables Ia and Ib).

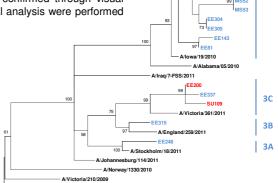
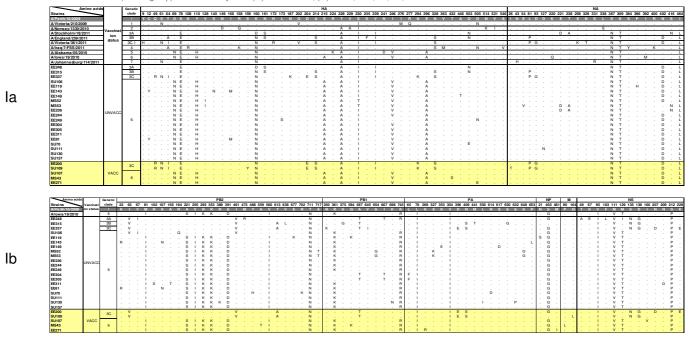


Figure 1 — Maximum likelihood phylogenetic tree of hemagglutinin and whole genome concatenated nucleotide sequences. Bootstrap values above 50 are shown (500 replicates). Sequenced viruses in blue (vaccinated individuals) and red (unvaccinated individuals). The vaccine strain is highlighted in yellow, and reference strains in black.

Table Ia and Ib - Amino acid substitutions observed in the 8 viral genome segments region of studied influenza A(H3) viruses comparing to the 2011/2012 vaccine strain A/Perth/16/2009. Viruses detected in unvaccinated individuals (control group) are shaded in yellow. (Ia) – HA and NA; (Ib) – PB2, PB1, PA, NP, M and NS.



Conclusions:

- In all 8 genomic segments of studied viruses no particular amino acid substitution was found to be associated with the vacc or unvacc cases. The observed differences were associated with the genetic distances between the clades to which viruses belong rather than with vaccine failure.
- WGS in influenza surveillance is a powerful tool for monitoring the overall evolution of viral genome and establishment of molecular markers for disease severity and drug resistance.
- This study points that a full evaluation of influenza vaccine failures should integrate not only data on virus characteristics, but also on host genetic polymorphisms related to immunity and serosurveys in order to better evaluate interindividual variation in influenza vaccine-induced immune responses.