Molecular epidemiology of the pandemic human Influenza A(H1N1)2009 in Belgium

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Introduction
Swine-origin pandemic human influenza A(H1N1)2009 has spread rapidly around the world since its initial reporting on 25 April 2009. The influenza A(H1N1)2009 virus is a unique combination of gene segments from both North American and Eurasian swine influenza viruses and has a high mean evolutionary rate for individual segments and the whole genome (3.66 × 10⁻³ substitutions per site per year) [Smith et al., 2009]. This study aims to gain insight into the genetic diversity of the A(H1N1)2009 strains which circulated in Belgium based on the viral external hemagglutinin (HA) gene.

Patients and methods
In Belgium, nationwide surveillance is conducted by the National Influenza Centre (NIC). A total of 139 general practitioners (GPs) distributed over the whole country participated in the virological and clinical surveillance of pandemic influenza. Nasopharyngeal samples were taken from patients presenting with influenza like symptoms (ILIs) from week 29/2009 until week 23/2010, and were analyzed by real-time RT-PCR. From week 29 throughout week 38, around 10% of the tested samples were found positive for influenza A(H1N1)2009. From week 42 until week 46, more than 50% of the samples were found positive for influenza A(H1N1)2009, corresponding to the peak of the epidemic in Belgium. From week 47 onwards prevalence lowered, and from week 53 the epidemic criteria were no longer fulfilled, with less than 141 ILI consultations per 100,000 inhabitants and less than 20% of the samples positive for influenza A(H1N1)2009.

Results
A series of randomly selected samples from the start, peak and end of the epidemic were sequenced. Total RNA was extracted from nasopharyngeal samples using the QIAmp Viral RNA extraction kit (Qiagen) and was amplified by in-house methods using the One-Step RT-PCR system (Invitrogen) to yield partial sequences of the hemaglutinin (HA) gene (1541nt). Sequencing was performed using an automated ABI Prism 3130 instrument by use of Big Dye 3.1 cycle sequencing kits (Applied Biosystems).

The sequences were aligned using MUSCLE [Edgar, 2004]. Phylogenetic analyses were performed using PhyML [Guindon, 2003]. A consensus tree was created using CONSENSE [Felsenstein, 1989] from 100 bootstrap replicates of maximum likelihood trees estimated by PhyML using the HK85 model.

Figure 1. Detection of influenza A/H1N12009 in nasopharyngeal samples from patients presenting to general practitioners with an influenza like illness during the 2009/2010 influenza pandemic.

Figure 2. Consensus phylogenetic tree of HA sequences (nt 1-1541) of selected A/H1N1/2009 viruses circulating in Belgium during the start, peak and end of the 2009-2010 flu epidemic. Node labels are bootstrap values.

We examined the nucleotide sequences (amplified from nasopharyngeal swabs) of the hemagglutinin gene (HA, bases 1-1541) from 25 influenza A(H1N1)2009 strains isolated in Belgium, in the period of May 2009 to March 2010. Figure 2 shows the consensus phylogenetic tree of these 25 HA sequences together with representative HA sequences of 7 strains found in other parts of the world.

Most clusters were not well supported, limiting our ability to draw conclusions based on molecular evolution. In this sample of Belgian isolates we could not confirm the global clusters found in Nelson et al. Although our consensus tree shows the same clustering pattern differentiated by the same 2 signature mutations at position 658 and position 1408 of the HA gene as in Fereidouni et al., this is only supported by a low bootstrap value of 38.

Conclusion
Our findings suggest the differentiation of influenza A(H1N1)2009 viruses circulating in Belgium into 2 distinct clusters among the circulating influenza A(H1N1)2009 viruses worldwide, contributing additional knowledge of the pandemic virus and encouraging further research into this topic.

References

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