

# Pandemic Influenza Virus Surveillance in Portugal: The Laboratory Network for the Diagnosis of Influenza A(H1N1)2009 Infection

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## Background

In April 2009 the World Health Organisation alerted for the circulation of a new strain of Influenza A(H1N1) virus of swine origin, which disseminated throughout the world resulting in the first Influenza pandemic of the XXI century. To face the increasing number of diagnosis being requested by the Health System, a Network of Laboratories trained in molecular diagnostic methodologies was activated in our country to perform the diagnosis of the new Influenza A(H1N1) pandemic virus.

The Laboratory Network for the Diagnosis of Influenza A(H1N1)2009 Infection is currently composed of 13 laboratories in Portugal including the Autonomous Regions of Madeira and Açores. The information collected through this Network complements the information collected through the National Influenza Surveillance Programme to give a clearer picture of the Influenza activity in Portugal during the 2009/2010 pandemic season.

This is a descriptive study of the pandemic Influenza activity in Portugal, based on the information collected through the Laboratory Network for the Diagnosis of Influenza A(H1N1)2009 Infection.

## Materials and Methods

### The Laboratory Network for the Diagnosis of Influenza A(H1N1)2009 Infection

Established in 2006 and reactivated and updated in June 2009 in the context of the 2009 Influenza pandemic, this Network is currently composed of 13 Laboratories from Reference Hospitals within mainland Portugal and the Islands:

ARS do Algarve, I.P. - Laboratório Regional de Saúde Pública Laura Ayres (LLA)  
Hospitais da Universidade de Coimbra, E.P.E. (HUC)  
Hospital Central do Funchal, EPE (HCF)  
Hospital de Curry Cabral (HCC)  
Hospital de S. João, E.P.E. (HSJ)  
Hospital de Santa Maria, E.P.E. (HSM)  
Hospital de Santo Espírito de Angra do Heroísmo, E.P.E. (HAH)  
Hospital do Divino Espírito Santo de Ponta Delgada, E.P.E. (HDES)  
Hospital do Espírito Santo de Évora, E.P.E. (HESE)  
Instituto de Medicina Molecular da FML da Universidade de Lisboa (IMM)  
Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. (INSA)  
Hospital Dona Estefânia (HDE)  
Centro Hospitalar do Porto - Hospital de Santo António (CHP)

The main objective of this Network is to carry out the laboratory diagnosis of Influenza A(H1N1)2009 infection on specimens collected through the National Health Service, thus contributing to the evaluation of the Influenza activity and characterisation of Influenza strains in circulation.

### Surveillance Period

This study describes the Influenza activity during the 2009/2010 Influenza season, based on the information collected through the Laboratory Network.

The surveillance of the Influenza activity in Portugal is carried out through the National Influenza Surveillance Programme, coordinated by the National Influenza Reference Laboratory, and is usually reactivated on September of one year (week 40) and follows through May of the following year (week 20). However, the Influenza period here described was epidemiological different from a "normal" influenza winter season. For this reason, it was decided to consider the 2009/2010 pandemic season from week 17/2009 to week 20/2010. This particular division was considered considering that the first Influenza case attributed to Influenza A(H1N1)2009 infection was observed on week 17/2009.

### Cases and variables studied

ILI cases were reported through a common web-based database from week 15/2009 through week 27/2010. Information collected included clinical and epidemiological data. Nasopharyngeal swabs were collected for virological characterisation of influenza viruses circulating during this period.

## Results

A total of 62089 ILI cases were reported through the Laboratory Network during the 2009/2010 pandemic season (Table I).

Two peaks were observed, one occurring towards the end of the Summer'09 and another during November-December (Figure 1).

Table I. Number of ILI cases reported by Network participants and Health Region. Geographic distribution of ILI should not be inferred from this data, distribution of ILI cases per Health Region is merely informative

Health Region	Laboratory Network participants	No. of ILI cases reported
North	Centro Hospitalar do Porto	397
	Hospitais São João	16185
Centre	Hospitais Universidade de Coimbra	985
	Hospital Curry Cabral	1409
Lisbon and Tejo Vale	Hospital Dona Estefânia	153
	Hospital Santa Maria	1466
	Instituto de Medicina Molecular	1169
	Instituto Nacional de Saúde Dr. Ricardo Jorge	16376
ALENTEJO	Hospital Espírito Santo	2836
ALGARVE	Laboratório Laura Ayres	11364
Açores	Hospital Angra do Heroísmo	3297
	Hospital Divino Espírito Santo	4589
MADEIRA	Hospital Central do Funchal	2063
Total		62089

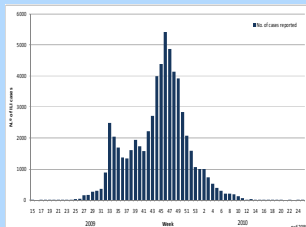


Figure 1. Distribution of ILI cases reported per week of onset of symptoms

The first Influenza A(H1N1)2009 infection in Portugal was detected during week 17/2009, when seasonal Influenza viruses, linked to the previous winter season, were still circulating. A total of 25985 laboratory confirmed Influenza cases were found (Figure 2), over 99% of which associated with the new pandemic strain A(H1N1)2009, with a peak occurring during week 33 with 1145 influenza cases, followed by a second peak during week 46 with 3266 influenza cases.

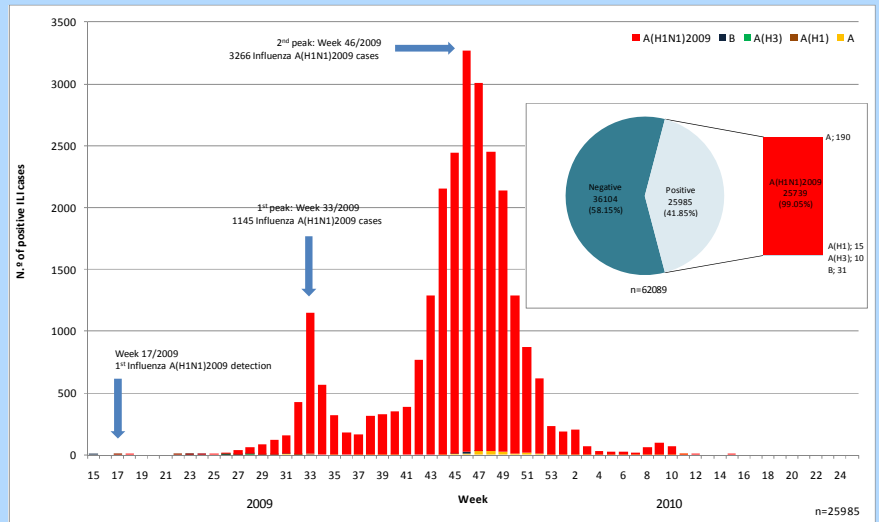


Figure 2. Total and weekly distribution of Influenza-positive ILI cases by Influenza virus type and subtype

Although the majority of notifications occurred in the adult population (aged 15-44 years) (21267), the highest percentage of Influenza-positive results was observed in children (aged 5-14 years) (64.0%) (Figure 3). The elderly recorded the lowest number of reports and Influenza-positive results.

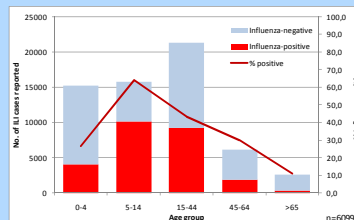


Fig. 3. Distribution of notifications and Influenza cases by age group. 1095 cases were excluded due to missing information on age.

Ninety five strains were isolated and antigenically characterised, 45 of which were also genetically analysed (HA and NA gene). All these strains were antigenically and genetically similar to the pandemic vaccine strain (Figure 4). The mutation H275Y, associated with resistance to oseltamivir was only found in one strain, which was proved to be resistant by phenotypic assays.

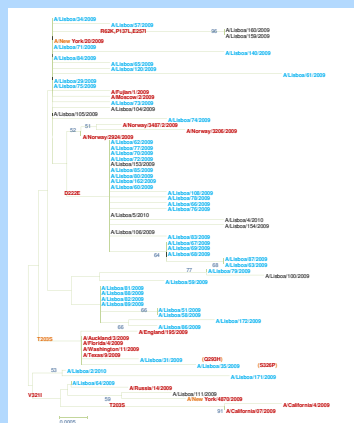


Fig. 4. Phylogenetic tree of HA1 nucleotide sequences from pandemic influenza A(H1N1)2009 strains isolated in Portugal (Neighbour-joining method; Kimura 2-parameter model; bootstrap 2000 replicates). Reference strains are indicated in red, strains obtained from the Laboratory Network are shown in blue, strains in black were obtained from the National Influenza surveillance Programme.

Table II shows the independent odd ratios for the association of each symptom/sign considered for the clinical definition of Influenza, according to the International Classification of Health Problems in Primary Care, and a confirmed Influenza case. It can be observed that, with the exception of respiratory difficulty, all symptoms/signs considered revealed an elevated risk of being associated with a confirmed Influenza diagnosis.

Table II. Association between the presence of the symptoms/signs considered for clinical definition of Influenza (according to the International Classification of Health Problems in Primary Care) and a confirmed Influenza case

Symptom/sign	% positive	Odds ratio	IC95%
Fever	44.1	1.3	(1.3; 1.4)
Headache	50.4	1.8	(1.8; 1.9)
Myalgia	47.2	1.4	(1.4; 1.5)
Cough	47.4	2.4	(2.3; 2.5)
Sore throat	42.1	1.0	(0.4; 2.4)
Respiratory difficulty	29.4	0.5	(0.4; 0.5)
Contact with another ILI patient	63.6	3.6	(3.1; 4.1)

## Comments

Facing the circulation of a new virus and the threat that this could impose to the population and to the health care system, the total number of ILI cases reported and analysed in our country during the 2009/2010 winter boosted to numbers not seen in previous Influenza seasons. In terms of pattern of disease, the data collected through the Laboratory Network suggests that the 2009/2010 pandemic was similar to the previous Influenza season in terms of intensity and geographic distribution. In terms of temporal distribution, the pandemic season was characterised by two waves of ILI cases reported, one towards the end of the summer and another during the winter. Seasonal viruses were replaced by the new Influenza A(H1N1)2009 strain, which caused disease particularly in young children.

Using the data generated from the Laboratory Network made it possible to better characterise the beginning of the pandemic season, as the early cases were mainly reported to the Reference Hospitals that were dedicated to the isolation of patients in an attempt to contain the virus and contribute to the management of the infection. During the mitigation phase of the pandemic, when the new pandemic strain had disseminated throughout the world, ILI cases were reported in parallel between the Laboratory Network and the National Influenza Surveillance Programme, which then allowed for the clinical, epidemiological and virological characterisation of the infection.