

# Influenza Virus Genotypes Circulating In Central Greece During 2012-2014 And Vaccine Strain Match

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## Abstract

During the period 2012-2014 in central Greece, influenza A(H1N1), A(H3N2) and B virus genotypes were detected and isolated from individuals with influenza illness. Influenza A(H1N1) and A (H3N2) viruses were the dominant virus type in circulation as it was detected in 9% and 12% of pharyngeal swabs examined by real-time RT-PCR during both seasons, respectively, while type B viruses were detected in only 3% of the samples examined. Influenza activity in central Greece, as was determined by number of reported influenza cases and influenza positive samples detected, was markedly increased in the 2013-14, as compared to the 2012-13 season. Influenza A(H1N1)pdm09 viruses were detected during the 2013-14 season along with A (H3N2) and type B viruses. All type A(H1N1), A (H3N2) and type B influenza isolates analyzed by cell culture reacted to a high titer (> 640) against antisera to vaccine-like viruses of the same period, indicating satisfactory influenza vaccine protection against circulating seasonal and pandemic influenza viral strains.

## INTRODUCTION

Influenza type A and B viruses possess a segmented RNA genome which promotes the periodic emergence of influenza viral variants responsible for influenza epidemics and significant disease burden in the community. (1, 2). The surface antigens of influenza viruses, the hemagglutinin (HA) and neuraminidase (NA) are under selective pressure from the host immune system, constituting the basic mechanism of antigenic drift evident in influenza viruses (3, 4)

Influenza surveillance programs aim to reduce the impact of influenza by efficient vaccination programs. Global influenza activity is associated with increased morbidity and mortality indices due mainly to complications that result from infection, especially among individuals belonging to the high-risk groups (2, 5). Yearly vaccination with the trivalent influenza vaccine is recommended at the beginning of the influenza season in order to reduce the impact of the disease in the community (7).

The present study reports laboratory data relating to influenza virus molecular epidemiology in central Greece and constitutes the first systematic survey of influenza viruses in central Greece.

## MATERIALS AND METHODS

### Clinical specimen

During the period 2012-14 a total of 865 pharyngeal swabs were collected from non-vaccinated individuals of all ages based on the gravity of symptoms associated with influenza-like illness (ILI), 3-4 days following the onset of symptoms, which included high fever (>38.50C), arthralgia, myalgia, cough, headache, general malaise. Sampling took place during the influenza season in Greece from December to April, each year by health professionals at the University Hospital and by physicians of the sentinel network, Larissa, Thessaly, Greece. Sample storage and transport was accomplished in virus transport medium (24,9 gr bacto-tryptose, 5 gr gelatin, 0,5 gr streptomycin, 500,000 U penicilin and 50 mg fungizone) per liter. Storage was at

+40C for up to 4 days. Long-term storage of the clinical samples was at -70oC.

### **Real-time RT-PCR (rt-RT-PCR)**

Total RNA was extracted from 200ml of processed clinical sample with TRIzol (Gibco BRL, Life Technologies, NY, USA), according to manufacturer's recommendations, in the presence of 20mg glycogen, molecular biology grade (Boehringer, Germany). Reactions in real-time RT-PCR were performed in a 7500 Applied Biosystem thermocycler, at a total reaction volume of 25µl (including 6.5µl RNA). Five different master mixes were prepared for each sample (influenza A, influenza B, H1, H3 and internal control). Forty reaction cycles were performed as follows: 450C for 10 sec, 950C for 10 min and 720C for 1min. Primers and probes used in the rt-RT-PCR reactions are shown in Table 1.

### **Virus isolation and identification**

Following screening by real-time RT-PCR, all positive samples were inoculated into sensitive Mardin-Darby Canine Kidney (MDCK) cell culture and incubated at 37oC for 7-10 days, as previously described (6 ). The Hemagglutination test was performed on culture supernatants with 0.5% red blood cells and a titer of > 32 was used for strain identification with the Hemagglutination-inhibition method using antisera and according to World Health Organization for virus standard protocol (7 ).

## **RESULTS**

During the period 2012-13, 342 respiratory samples were examined at the microbiology laboratory of the University Hospital, Larissa, Greece. Following initial screening with rt RT-PCR assay, 80 samples (23%) were found positive, of those 14% were positive to influenza type A (H3N2) viruses and 9% to influenza type B viruses. There were no A (H1N1) viruses detected during the same period from the samples examined. In the 2013-14 season 523 samples were examined and 139 (27%) were found positive by rt RT-PCR. During the 2013-14 season, influenza type A (H3N2) and A (H1N1) and type B viruses were detected and isolated from the positive samples. More specific, of the positive samples of the 2013-14 season, 98% tested positive for type A viruses and only 2% for type B viruses. Influenza A (H1N1) and A (H3N2) viruses were detected in 60% and 40% of the positive type A viruses, respectively, (Table 2).

Analysis of the hemagglutinin antigen of the A (H1N1) and

A (H3N2) and B viruses by the HAI method following virus propagation in MDCK cells revealed relatedness (HAI titer of >1/640) to the WHO influenza vaccine strains of the respective periods, (data not shown). Similarly, comparison of the hemagglutinin nucleotide sequences of A (H1N1) and A (H3N2) viruses with those of reference and vaccine influenza strains revealed homology of 98% (data not shown). In all the samples examined during the above mentioned periods there were found no mixed infections, type A or B. Also, we found 100% correlation between the rt RT-PCR and cell culture results.

## **DISCUSSION**

Based on our findings, it is shown that following the global circulation of pandemic A (H1N1) viruses during the 2009-11 period, influenza A (H1N1) virus activity in central Greece was at very low levels during the 2012-13 season as it was not detected in any of the samples examined. This finding is consistent with the epidemiology of A (H1N1) viruses as reported by other countries of the WHO network (8-10). In comparison to the vaccine strains of the 2012-2013 and later seasons, our A (H1N1) viral isolates were antigenically similar to the A/California/7/2009 (H1N1) pdm09 viruses while our A (H3N2) isolates reacted to a high titer against the A/Victoria/361/2011(H3N2)-like viruses, indicating that there was provided adequate immune coverage by the influenza vaccination program during these seasons.

Our findings also suggest that during the 2012-2014 period, influenza A viruses were the dominant viral type to circulate in different regions of central Greece, showing antigenic and molecular similarity to vaccine-like viruses also in circulation during the same period in different parts of the world. This work constitutes a preliminary study on influenza molecular epidemiology in central Greece and further underlines the importance of regular influenza epidemiological and virological surveillance in order to more effectively manage yearly influenza activity.

**Table 1**

Primers and probes used in real-time RT-PCR

**Influenza type A**

A-Fw CTTCTAACCGAGGTCGAAACGTA  
 A-Rv GGTGACAGGATTGGTCTTGTCTTTA  
 A-probe- 5'-Fam -TCAGGCCCCCTCAAAGCCGAG-3'

**Influenza type B**

B-Fw ATTGCTGGTTTCTTAGAAGG  
 B-Rv TTGTTTATRGCCTTCTTGMGT  
 B-probe 5'-Fam- ATGGGAAGGAATGATTGCAGGT-3'

**A (H1N1)**

H1-Fw GAGCTAAGAGAGCAATTGA  
 H1-Rv GTAGATGGATGGTGAATG  
 H1- Probe 5'-Fam -TTGCTGAGCTTTGGGTATGA -3'

**A (H3N2)**

H3-Fw AGCAAAGCCTACAGCAA  
 H3-Rv GACCTAAGGGAGGCATAA  
 H3-Probe 5'-Fam-CCGGCACATCATAAAGGGTAACA 3'

**Table 2**

Influenza virus genotypes circulating in central Greece during 2012-2014

Influenza Season	2012-13	2013-14	Total
Samples examined	342	523	865
A (H1N1)	0	81 (16%)	81 (9%)
A (H3N2)	49 (14%)	55 (11%)	104 (12%)
B	31 (9%)	3 (< 1%)	33 (3%)

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