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# Detection and typing of influenza viruses in vaccinated and non- vaccinated children in central Greece during 2018-2019

## Abstract

During the 2018-2019 influenza season in central Greece, 388 respiratory samples were collected from children presented with influenza symptoms and tested for the detection of influenza viruses. Real-time RT-PCR testing of the samples revealed the presence of influenza A (H1N1) (pdm09), influenza A (H3N2), seasonal influenza A (H1N1), and influenza B viruses. All samples tested were found negative for influenza A(H5) HA gene sequence. The majority of influenza viruses detected were type A (90%), of which the majority, (61%), were influenza A (H1N1) (pdm09) viruses, indicating that these viruses were the dominant influenza subtype circulating in central Greece during the winter and early spring of the aforementioned season.

**Keywords:** influenza viruses, laboratory diagnosis, central Greece

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## Introduction

Influenza viruses, being the etiologic agents of acute epidemic respiratory illness, contribute yearly significant disease burden on individuals of all ages, and primarily on children and the elderly. Influenza virus types A and B co-circulate and have been detected and isolated from clinical samples in many geographic regions of the world, yearly.[1] The epidemiological success of influenza viruses is due primarily to the polymorphism in their external antigens, hemagglutinin and neuraminidase. Such changes, frequently result in the emergence of novel virus variants against which there is insufficient immunity in the population, resulting in yearly disease outbreaks, globally.[2–4] Increasingly, over the last two decades, many virology and public health laboratories perform routine molecular testing and analysis of the HA and NA gene segments along with conventional virus isolation and antigenic typing methods, thereby improving influenza disease management and vaccine efficacy.[3,5] In this study, we report the results from assaying respiratory samples from vaccinated and non-vaccinated children, presenting influenza symptoms, during the winter 2018-2019, for influenza types A (H1N1) (pdm09), A (H3N2), A (H1N1), and type B viruses, using cell culture and real-time RT-PCR methods. Our study constitutes the only laboratory diagnosis of influenza viruses in central Greece for the concerning period.

## Material and methods

### Clinical samples

A total of 388 naso-pharyngeal swabs were collected during the period from December 2018 to April 2019, as follows: 188 from non-vaccinated and 200 from vaccinated children ages 0-15, during visits to private practice pediatricians in the region of central Greece. Samples were collected on the basis of the presence of at least one of the following influenza symptoms: fever >38.5$^0$C, malaise, mild to severe headache, mild to severe sore throat, cough, and myalgia, 3-4 days after the onset of symptoms. All samples were collected into vials containing 2.5 ml virus transport medium (24,9 gr bacto-tryptose, 5 gr gelatin, 0,5 gr streptomycin, 500000 U penicilin and 50 mg fungizone per liter) and stored at +4$^0$C until testing.

### Cell culture

A selected number of samples were propagated in cell culture. Prior to cell culture, samples were centrifuged in aliquots at 1200 rpm for 5min. and stored at +4$^0$C for up to 4 days or at -80$^0$C for longer period. Processed clinical samples in virus transport medium were transferred into microtubes and 125µl of the supernatant was inoculated into confluent MDCK, which are sensitive to infection by human influenza viruses including the A(H1N1) pdm09 strain. The cell culture medium contained Dulbecco's Modified Eagle medium (D-MEM), 10% fetal calf serum (Gibco), 100 IU/ml penicillin and 100 mg/ml streptomycin and 1% trypsin (Sigma, USA). MDCK cultures were incubated 370C for up to 7 days and were observed daily for the appearance of cytopathic effect (CPE). The hemagglutination test was performed on culture supernatants using 0.5% chicken red blood cells to detect the presence of HA antigen, including control antigens, according to standard WHO protocols.[6]

### One-step real-time RT-PCR

In order to perform One-Step RT-PCR, viral RNA was extracted from 200 µL of virus transport medium using GRS Viral DNA/RNA Purification Kit (GriSP, Portugal), according to manufacturer's instructions. In the one-step Real-time RT-PCR, all samples were initially screened for the presence of influenza A and B viral seuences, followed by subtyping reactions for A (H1N1) (pdm09), H3 and seasonal influenza A (H1N1) viruses. Additionally, all samples were screened for the detection of influenza A(H5) HA gene. 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) using Invitrogen Super script® III Platinum® one-step qRT-PCR kit. In every one-step Real-time RT-PCR reaction, there was included a positive control of known titer for each virus type and subtype, negative extraction control and MS2 Phage Control RNA to verify the extraction procedure and the absence of inhibitors in the PCR reaction. The primer sequences, and the one-step Real-time qRT-PCR protocol were according to the WHO information protocol for the molecular detection of influenza viruses.

## Results and discussion

### Influenza virus detection

During the 2018-2019 winter season, 388 respiratory samples originating from vaccinated and non-vaccinated children were examined for the presence of influenza viruses with real time one step Real-time qRT-PCR. Positive for influenza were found 365 samples (94%), of those 347 (95%), were from non-vaccinated and 17 (5%), from vaccinated children (Table 1 & 2).

**Table 1** Real time RT-PCR testing for influenza A and B viruses

| Total Number of samples | Positive for Influenza A/B | Negative for Influenza A/B |
|---|---|---|
| 388 | 365 | 23 |
| | 94,07% | 5,93% |

**Table 2** Vaccinated/ non-vaccinated positive samples

| Total positive | Vaccinated | Non vaccinated |
|---|---|---|
| 365 | 347 | 17 |
| | 95,06% | 4,94% |

### Influenza virus genotyping by real-time RT-PCR

Positive to influenza A were found 328 samples (89, 87%), and 37 for influenza B virus (10,13%), (Table 3). Subtyping of the influenza positive samples revealed that 200 (61%) were type A (H1N1) (pdm09), 121 (37%) were type A (H3N2), and 7 (2%) were positive for seasonal type A (H1N1) viruses. Additionally, there were found no positive samples for the influenza A(H5) HA gene (Table 4). The laboratory results for influenza viruses on the respiratory pediatric samples assayed coincide with the epidemiological and virological data, from influenza surveillance in other countries of the northern hemisphere, during the 2018-19 season, where the A (H1N1) (pdm09) viruses co-circulated with the A (H3N2) viruses and were detected in the majority of clinical samples examined.[7,8]

**Table 3** Influenza virus genotyping

| Total positive | Influenza A | Influenza B |
|---|---|---|
| 365 | 328 | 37 |
| | 89,87% | 10,13% |

**Table 4** Influenza a virus subtyping

| Influenza A positive | influenza A (H1N1) (pdm09) | influenza A (H3N2) | Seasonal influenza A (H1N1) | influenza A(H5) |
|---|---|---|---|---|
| 328 | 200 | 121 | 7 | 0 |
| | 61% | 37% | 2% | 0% |

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None.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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