Influenza Virus Activity During Pandemic And Seasonal Influenza In Central Greece

E Plakokefalos, A Vontas, Z Florou, G Valiakos, K Georgantza, S Sarrou, M Labronikou, E Gramoustianou, E Peteinaki, P Markoulatos, A Minas, V Krikelis, A Noulas, A Manouras

Citation

E Plakokefalos, A Vontas, Z Florou, G Valiakos, K Georgantza, S Sarrou, M Labronikou, E Gramoustianou, E Peteinaki, P Markoulatos, A Minas, V Krikelis, A Noulas, A Manouras. *Influenza Virus Activity During Pandemic And Seasonal Influenza In Central Greece*. The Internet Journal of Infectious Diseases. 2015 Volume 14 Number 1.

DOI: 10.5580/IJID.33808

Abstract

In central Greece, during the influenza seasons from 2009-2012, type A influenza A (H1N1) viruses were the dominant subtype to circulate in the population. This viral subtype was detected in 100% of the positive samples assayed, during the 2009-10 and 2010-11 seasons, respectively. This finding coincides with the emergence of pandemic influenza A (H1N1) viruses, globally. The A (H1N1) viruses detected were identified as antigenically similar to the vaccine A (H1N1) pdm 09 viral strains of the respective periods. Overall, with the exemption of the 2011-12 season, where only influenza A (H3N2) and influenza B viruses were detected, in 61% and 39% of the samples assayed by real-time RT-PCR, respectively, A (H1N1) pdm 09 viral strains were isolated from 94% of the positive by real-time RT-PCR samples. These findings support the importance of continuous influenza laboratory surveillance and influenza vaccine formulation according to the antigenic profile of circulating viral strains.

INTRODUCTION

Influenza virus types A and B viruses are the etiologic agents of acute epidemic respiratory infection. Type A viruses are classified into subtypes based on the constitution of their hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins (1, 2). Currently there are classified 16 HA and 9 NA different antigenic subtypes circulating in different animal species including humans (3). Influenza viruses have frequently been associated with yearly epidemic episodes due to high rate of evolution of their HA and NA molecules (4).

Each year influenza and complications that result from infection account for increased indices of morbidity and mortality worldwide, especially among individuals belonging to the high-risk groups. 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 (5). In March 2009, an A (H1N1) virus emerged with a pandemic potential, containing a combination of avian, human and swine gene segments, and quickly spread worldwide, exhibiting both increased pathogenicity and resistance to the antiviral oseltamivir (6).

In the present study, we report the laboratory findings associated with influenza virus detection and identification during the influenza seasons from 2009-2012.

MATERIALS AND METHODS

Pharyngeal swabs

A total of 4156 respiratory specimen were collected during the period 2009-12 from individuals of all ages presenting with influenza like illness (ILI), 3-4 days following the onset of symptoms at the University Hospital, Larissa, Greece. Symptoms included fever (>38.50C), malaise, headache, myalgia and cough. All samples were collected into 2.5 ml 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). Samples were examined immediately or stored at +40C for up to 4 days.

Real-time 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 reactions are shown in Table 1.

Virus isolation in cell culture and virus identification

Selected real-time RT-PCR positive samples were grown in Mardin-Darby Canine Kidney (MDCK) cells at 37oC for 7-10 days. The hemagglutination test was performed on culture supernatants and a titer of 1/32 or higher was used for strain identification with the hemagglutination-inhibition method as previously described(7). Antisera for virus identification was used according to World Health Organization protocol (8).

RESULTS AND DISCUSSION

Influenza virus genotyping

During the period 2009-2012, 4156 respiratory samples were collected and examined at the microbiology laboratory of the University Hospital, Larissa, Greece. Analytically, there were examined by real-time RT-PCR 2319, 1423, and 414 samples in 09-10, 10-11, and 11-12, respectively. Out of the total number of samples, 1430 (34%) were found positive for influenza virus type A or B. There were found no mixed infections in the positive samples. Specifically, in the 09-10 and 10-11 seasons, where there was an outbreak of pandemic influenza in central Greece, as reported by regional sentinel network of physicians, 3814 samples were assayed and 1350 (35%) were found positive. In these two seasons 100% of the positive to influenza samples were A (H1N1) viruses, no A (H3N2) and type B viruses were detected. Analytically, during the 09-10 season 2319 samples were assayed and 1075 (46%) were found positive and in the 10-11 season, 1443 samples were assayed and 275 (19%) were found positive (Tables 2 and 3). Influenza A (H3N2) and influenza B viruses were detected in the 11-12 season, where 342 samples were assayed and 80 (23%) were positive, of those 49 (61%) were A (H3N2) and 31 (39%) type B viruses, there were no A (H1N1) viruses detected in the samples examined in the latter season (Table 4).

Overall, in comparing type A virus detection during the three seasons, 32% were A (H1N1) and 1% A (H3N2) (Table 5). Identification of the pandemic viral strains, from the 09-10 and 11-12 seasons, revealed antigenic similarity to the pandemic vaccine-like viruses (data not shown). Our findings suggest that the majority of influenza infections in central Greece during 09-10 and 10-11 seasons were due to antigenic variants of the pandemic virus A (H1N1) as is revealed in similar reports in neighboring regions and countries during the same period (9-12).

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 TTGTTTATRGCTTCTTGMGT
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-CCGGCACATCATAAGGGTAACA 3'

Table 2

Influenza virus detection during the 2009-2010 season

	Influenza A							Influenza B	
09-10	H1N1					H3N2		B	
	Samples	positive	%	negative	%	positive	%	positive	%
Total	2319	1075	46	--1244	54	--0	0	--0	0

Table 3

Influenza virus detection during the 2010-2011 season

	Influenza A							Influenza B	
10-11	H1N1					H3N2		B	
	Samples	positive	%	negative	%	positive	%	positive	%
Total	1443	275	19	1168--	81	--0	0	0--	

Table 4

Influenza virus detection during the 2011-2012 season

	Influenza A							Influenza B	
11-12	H1N1					H3N2			
	Samples	positive	%	negative	%	positive	%	positive	%
Total	342	0	0	342	100	-49-	61	--31	39

Table 5

H1N1.and H3N2 viruses detected during 2009-2012

	H1N1			H3N2	
2009-12					
	samples	positive	%	positive	%
Total	4156	1350	32	49	1

ACKNOWLEDGEMENTS

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

References

1. Lamb, RA, Krug RM, "Orthomyxoviridae: The viruses and their replication' In 'Virology' (Fields, BN, Knipe, DM, and Howley PM., Eds.), pp. 1353-1395. Lipincott-Raven, Philadelphia, 1996.
2. Cox, NJ, Subbarao K, 'Influenza' The Lancet. 354: 1277-1282, 1999.
3. Fouchier RA, Munster V, Wallenstein A, Bestebroer TM, Herfst S, Smith D, Rimmelzwann GF, Olsen B, Osterhaus AD, 'Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls'. J Virol 79:2814-2822, 2005.
4. Gamblin SJ, Skehel JJ, 'Influenza hemagglutinin and neuraminidase membrane glycoproteins'. J Biol Chem 285: 28403-28409, 2010.
5. Dawood FS, Jain S, Finelli L, Shaw M, Lindstrom S, Garten J, Gubareva LV, Xu X, Bridges CB, Uyeki TM, 'Emergence of a novel swine-origin influenza A (H1N1) virus in humans'. N Engl J Med 360:2605-2615, 2009.
6. Schnitzler SU, Schnitzler P, 'An update on swine-origin influenza virus A/H1N1: a review'. Virus Genes 39:279-292. 2009.
7. Plakokefalos ET, Vontas A, Gramoustianou E, Markoulatos P, Krikelis V, 'Evolution of hemagluttinin gene in archival influenza A (H3N2) viruses isolated in Southern Greece during 1980-2004'. Proceedings of the Scientific Conference pp:365-67, June, 10-14. 2013.
8. World Health Organization, 'Manual for the laboratory diagnosis and virological surveillance of influenza', WHO, 2011.
9. World Health Organization, Weekly Epidemiological Record, vol. 84 49 (505-516), 2009.
10.World Health Organization, Weekly Epidemiological Record, vol. 85 46 (453-460), 2010.
11.World Health Organization, Weekly Epidemiological Record, vol. 86 45 (497-508), 2011.
12.World Health Organization, Weekly Epidemiological Record, vol. 87 47 (461-476), 2012.

Author Information

E. Plakokefalos
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece
plakoke@yahoo.com

A. Vontas
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

Z. Florou
Department of Medicine, School of Health Sciences, University of Thessaly
Larissa, Greece

G. Valiakos
Department of Medicine, School of Health Sciences, University of Thessaly
Larissa, Greece

K. Georgantza
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

S. Sarrou
Department of Medicine, School of Health Sciences, University of Thessaly
Larissa, Greece

M. Labronikou
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

E. Gramoustianou
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

E. Peteinaki
Department of Medicine, School of Health Sciences, University of Thessaly
Larissa, Greece

P. Markoulatos
Department of Biochemistry and Biotechnology, University of Thessaly
Larissa, Greece

A. Minas
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

V. Krikelis
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

A. Noulas
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece

A. Manouras
Department of Medical Laboratories, Technological Educational Institute of Thessaly
Larissa, Greece