Influenza Virus Activity During Pandemic And Seasonal Influenza In Central Greece


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

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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 370C 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

<table>
<thead>
<tr>
<th>Primers and probes used in real-time RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Influenza type A</strong></td>
</tr>
<tr>
<td>A-Fw CTICTAACCGAGGTCAAAACGTA</td>
</tr>
<tr>
<td>A-Rv GGTGACAGGAATTGGTCTGGTCTTTA</td>
</tr>
<tr>
<td>A-probe 5'-Fam- TCAGGCCCCCTCAAGGCCAG-3'</td>
</tr>
<tr>
<td><strong>Influenza type B</strong></td>
</tr>
<tr>
<td>B-Fw ATTCGCTGTTCTTAGAAGG</td>
</tr>
<tr>
<td>B-Rv TGTITATATCGTCTCTGGMT</td>
</tr>
<tr>
<td>B-probe 5'-Fam- ATGGGAAGAGGATGACTGACGGT-3'</td>
</tr>
</tbody>
</table>

**A (H1N1)**

H1-Fw GAGCTAAGAGACATGGAATG
H1-Rv GTAGA1GGATGGTAATG
H1- Probe 5'-Fam -TTGCTGAGCTTTGGGTATGA -3'

**A (H3N2)**

H3-Fw AGCAAGCCCTACAGCAA
H3-Rv GACCTAAGGGAGCAATA
H3-Probe 5'-Fam-CCGGCACATCATAAGGGTAAAC 3'

Table 2

Influenza virus detection during the 2009-2010 season

<table>
<thead>
<tr>
<th></th>
<th>09-10 Samples</th>
<th>Influenza A %</th>
<th>Influenza B %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>H1N1</td>
<td>2319</td>
<td>1075</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 3

Influenza virus detection during the 2010-2011 season

<table>
<thead>
<tr>
<th></th>
<th>10-11 Samples</th>
<th>Influenza A %</th>
<th>Influenza B %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>H1N1</td>
<td>1443</td>
<td>275</td>
<td>19</td>
</tr>
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</table>
Table 4
Influenza virus detection during the 2011-2012 season

<table>
<thead>
<tr>
<th>H1N1</th>
<th>H3N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>positive</td>
</tr>
<tr>
<td>Total</td>
<td>342</td>
</tr>
</tbody>
</table>

Table 5
H1N1 and H3N2 viruses detected during 2009-2012

<table>
<thead>
<tr>
<th>2009-12</th>
<th>H1N1</th>
<th>H3N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>samples</td>
<td>positive</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>4156</td>
<td>1350</td>
</tr>
</tbody>
</table>
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