Characterisation of Influenza B Viruses Circulating in Portugal during the 2010/2011 Influenza Season

Pedro Pechirra, Patrícia Conde, Carla Romero, Carlos Ribeiro, Paulo Gonçalves, Raquel Guimarães

1Laboratório Nacional de Referência para o Vírus da Gripe, 2Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal

Objectives:
The continuous monitoring of the antigenic and genetic properties of circulating influenza viruses is essential in order to detect any new vaccine candidates or changes in current recommendations. During the 2010-2011 season, the influenza activity in Portugal was characterized by the co-circulation of influenza B-Victoria and pandemic A(H1N1)/2009 viruses with sporadic detections of influenza A(H3N2) and B/Yamagata viruses. This study reports the antigenic and genetic characterisation of influenza B viruses isolated in Portugal during the 2010/2011 influenza season.

Methods:
During the 2010/11 influenza season, 1017 nasopharyngeal swabs were collected from patients showing symptoms of influenza-like illness in the context of virological surveillance. The clinical specimens were collected by General Practitioners participating in the National Influenza Surveillance Programme and were tested at the National Influenza Reference Laboratory for the presence of influenza virus (RT-PCR). Seventy-one B/Victoria positive specimens were inoculated on MDCK cell cultures and the isolates were characterised antigenically by haemagglutination-inhibition assays (Manual for the Laboratory Diagnosis and Virology Surveillance of Influenza, 2011). Nineteen influenza B/Victoria (18 isolates and 1 clinical specimen designated by E8E) and two influenza B/Yamagata (one isolate and one clinical specimen designated by E6E22) viruses were selected for sequence analysis of the HA1 gene product (Pechirra et al., 2005). Phylogenetic analysis was performed using MEGA Software 5.05, phylogenetic trees were obtained by the Maximum Likelihood algorithm using Tamura-Nei nucleotide substitution model (Tamura et al., 2011).

Results:
Isolated influenza B/Victoria viruses showed high reactivity with serum raised against the vaccine strain B/Brisbane/60/2008 (Table I). An amino acid substitution that may justify the selection of different vaccine candidates or changes in current recommendations. During the 2010-2011 season, the influenza activity in Portugal was characterized by the co-circulation of influenza B-Victoria and pandemic A(H1N1)/2009 viruses with sporadic detections of influenza A(H3N2) and B/Yamagata viruses. This study reports the antigenic and genetic characterisation of influenza B viruses isolated in Portugal during the 2010/2011 influenza season.

Methods:
During the 2010/11 influenza season, 1017 nasopharyngeal swabs were collected from patients showing symptoms of influenza-like illness in the context of virological surveillance. The clinical specimens were collected by General Practitioners participating in the National Influenza Surveillance Programme and were tested at the National Influenza Reference Laboratory for the presence of influenza virus (RT-PCR). Seventy-one B/Victoria positive specimens were inoculated on MDCK cell cultures and the isolates were characterised antigenically by haemagglutination-inhibition assays (Manual for the Laboratory Diagnosis and Virology Surveillance of Influenza, 2011). Nineteen influenza B/Victoria (18 isolates and 1 clinical specimen designated by E8E) and two influenza B/Yamagata (one isolate and one clinical specimen designated by E6E22) viruses were selected for sequence analysis of the HA1 gene product (Pechirra et al., 2005). Phylogenetic analysis was performed using MEGA Software 5.05, phylogenetic trees were obtained by the Maximum Likelihood algorithm using Tamura-Nei nucleotide substitution model (Tamura et al., 2011).

Results:
Isolated influenza B/Victoria viruses showed high reactivity with serum raised against the vaccine strain B/Brisbane/60/2008 (Table I). An amino acid substitution that may justify the selection of different vaccine candidates or changes in current recommendations. During the 2010-2011 season, the influenza activity in Portugal was characterized by the co-circulation of influenza B-Victoria and pandemic A(H1N1)/2009 viruses with sporadic detections of influenza A(H3N2) and B/Yamagata viruses. This study reports the antigenic and genetic characterisation of influenza B viruses isolated in Portugal during the 2010/2011 influenza season.

Methods:
During the 2010/11 influenza season, 1017 nasopharyngeal swabs were collected from patients showing symptoms of influenza-like illness in the context of virological surveillance. The clinical specimens were collected by General Practitioners participating in the National Influenza Surveillance Programme and were tested at the National Influenza Reference Laboratory for the presence of influenza virus (RT-PCR). Seventy-one B/Victoria positive specimens were inoculated on MDCK cell cultures and the isolates were characterised antigenically by haemagglutination-inhibition assays (Manual for the Laboratory Diagnosis and Virology Surveillance of Influenza, 2011). Nineteen influenza B/Victoria (18 isolates and 1 clinical specimen designated by E8E) and two influenza B/Yamagata (one isolate and one clinical specimen designated by E6E22) viruses were selected for sequence analysis of the HA1 gene product (Pechirra et al., 2005). Phylogenetic analysis was performed using MEGA Software 5.05, phylogenetic trees were obtained by the Maximum Likelihood algorithm using Tamura-Nei nucleotide substitution model (Tamura et al., 2011).

Results:
Isolated influenza B/Victoria viruses showed high reactivity with serum raised against the vaccine strain B/Brisbane/60/2008 (Table I). An amino acid substitution that may justify the selection of different vaccine candidates or changes in current recommendations. During the 2010-2011 season, the influenza activity in Portugal was characterized by the co-circulation of influenza B-Victoria and pandemic A(H1N1)/2009 viruses with sporadic detections of influenza A(H3N2) and B/Yamagata viruses. This study reports the antigenic and genetic characterisation of influenza B viruses isolated in Portugal during the 2010/2011 influenza season.

Methods:
During the 2010/11 influenza season, 1017 nasopharyngeal swabs were collected from patients showing symptoms of influenza-like illness in the context of virological surveillance. The clinical specimens were collected by General Practitioners participating in the National Influenza Surveillance Programme and were tested at the National Influenza Reference Laboratory for the presence of influenza virus (RT-PCR). Seventy-one B/Victoria positive specimens were inoculated on MDCK cell cultures and the isolates were characterised antigenically by haemagglutination-inhibition assays (Manual for the Laboratory Diagnosis and Virology Surveillance of Influenza, 2011). Nineteen influenza B/Victoria (18 isolates and 1 clinical specimen designated by E8E) and two influenza B/Yamagata (one isolate and one clinical specimen designated by E6E22) viruses were selected for sequence analysis of the HA1 gene product (Pechirra et al., 2005). Phylogenetic analysis was performed using MEGA Software 5.05, phylogenetic trees were obtained by the Maximum Likelihood algorithm using Tamura-Nei nucleotide substitution model (Tamura et al., 2011).

Conclusions:
The majority of influenza B viruses characterised in Portugal during the influenza season 2010/2011 were found similar, both antigenically and genetically, to the vaccine strain B/Brisbane/60/2008. Nucleotide sequence analysis revealed 2 different genetic subgroups within the B/Brisbane/60/2008 genetic clade. Isolated influenza B/Victoria viruses revealed only one amino acid substitution in antigenic sites of the HA molecule comparing with vaccine strain (Wang et al., 2008). The influenza B/Yamagata viruses characterised grouped with the reference strains B/Wisconsin/1/2010 as did the majority of B/Yamagata viruses detected in circulation worldwide this season.

Acknowledgments:
The authors would like to thank Dr. John McCauley and the staff of the WHO Collaborating Centre (NMR, London) for providing the results of the detailed antigenic analysis of the Portuguese strains.

Bibliography:

Table I – Antigenic characterisation of 30 isolated influenza B/Victoria strains (haemagglutination inhibition test). Strains generally characterised are isolated in yellow.

<table>
<thead>
<tr>
<th>Strain</th>
<th>HA1</th>
<th>HA2</th>
<th>NA</th>
<th>NP</th>
<th>M</th>
<th>PB1</th>
<th>PB2</th>
<th>PB2</th>
<th>NS1</th>
<th>NS2</th>
<th>S1</th>
<th>S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E8E</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>E6E22</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Table II – Amino acid substitutions observed in the HA1 subtype of influenza B/Victoria viruses comparing to the vaccine strain B/Brisbane/60/2008. Antigenic sites of the hemagglutinin molecule are represented in red.

<table>
<thead>
<tr>
<th>Site</th>
<th>Amino Acid</th>
<th>2010/2011</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>R</td>
<td>2010/2011</td>
<td>Vaccine</td>
</tr>
</tbody>
</table>

For further information on this poster please contact: Requel Guimarães, Laboratório Nacional de Referência para o Vírus da Gripe, requel.guimaraes@lnsa.mt-saude.pt; Tel: 083951275012916, Fax: 08395127524900, Poster no. PD31

11th Annual Meeting of European Society for Clinical Virology, Florence, Italy, 25-28 September 2011