Journal of Clinical Virology 36 (2006) 208–214

www.elsevier.com/locate/jcv

# The reappearance of Victoria lineage influenza B virus in Brazil, antigenic and molecular analysis

F.C. Motta [a,*], M.M Siqueira [a], A.K. Lugon [a], S.M. Straliotto [b], S.B. Fernandes [c], M.M. Krawczuk [a]

[a] *Laboratório de Vírus Respiratórios e do Sarampo, Depto. de Virologia, Instituto Oswaldo Cruz, FIOCRUZ, 21045-900, Rio de Janeiro, RJ, Brasil*

[b] *LACEN-RS, Seção de Virologia, 90610-000, Porto Alegre, RS, Brasil*

[c] *LACEN-SC, Seção de Virologia, 88015-201, Florianópolis, SC, Brasil*

Received 5 July 2005; received in revised form 16 March 2006; accepted 21 March 2006

**Abstract**

*Background:* In contrast to influenza A, minor influenza B viruses can co-circulate with the dominant strain during an epidemic allowing the re-emergence of old strains and reassortment between those different strains. The 2001–2002 influenza season in the northern hemisphere was distinguished by the re-emergence of the Victoria-lineage viruses, which replaced the Yamagata-lineage, after being restricted to East Asia throughout the 1990s.

*Objectives:* To describe the antigenic and genetic characteristics of influenza B viruses detected in South and South East Brazil and determine their lineages.

*Study design:* Influenza samples collected during epidemics between 1999 and 2002 were analyzed by indirect immunofluorescence assay (IFA). Positive results were confirmed through multiplex PCR and isolation in cell culture. Isolated viruses were antigenically characterized by hemagglutination inhibition. Fourteen hemagglutinin (HA) gene sequences obtained in this work were used for phylogenetic analysis.

*Results:* Brazilian isolates from 2002 were associated with the Victoria-lineage, diverging from the vaccine used throughout that influenza season in Brazil.

*Conclusions:* These results indicate the reappearance of Sichuan/7/97-like samples in South and South East Brazilian Regions simultaneously. They indicate the need for neuraminidase gene evaluation and demonstrate the importance of influenza laboratory surveillance to establish which strains should be included in the influenza vaccine.



*Keywords:* Influenza B virus; Hemagglutinin inhibition; Phylogenetic analysis

## 1. Introduction

Influenza type B viruses are variable RNA viruses belonging to the family Orthomyxoviridae, with a single-strand negative sense segmented genome (Lamb and Krug, 1996). Although influenza B viruses have neither non-human reservoirs (except for a seal strain isolated in the Netherlands (Osterhaus et al., 2000)), nor distinct subtypes as observed in type A viruses (Rohm et al., 1996), they can be considered even more heterogeneous than type A, as antigenically and phylogenetically distinct lineages can co-circulate for long periods in the population (Barr et al., 2003). During the 1990s, most strains isolated worldwide were derived from B/Yamagata/16/88 (Yamagata-lineage), so strains from this lineage have been present in the influenza vaccines since the beginning of that decade (Anonymous, 1989). Strains derived from B/Victoria/2/87 lineage, circulated globally in the 1980s and made sporadic reappearances in Eastern Asia during the 1990s. In 2001, however, they re-emerged worldwide and

* Corresponding author. Tel.: +55 21 2598 4418; fax: +55 21 2573 9591.
*E-mail address:* fcmotta@ioc.fiocruz.br (F.C. Motta).

1386-6532/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2006.03.008

co-circulated with Yamagata-lineage strains. Strains were isolated in North America, China, Japan, Europe and Oceania (Barr et al., 2003; Shaw et al., 2002; Rimmelzwaan and Osterhaus, 2002). In addition to the genetic difference, the lack of cross-reactivity between antibodies for these two lineages (Kanegae et al., 1990) led WHO to recommend the use of Victoria-lineage strain, B/Shangdong/7/97, in the vaccine administered in Eastern Asia throughout the 1999–2000 epidemic (Anonymous, 1999). In this study, we describe the reappearance of the Victoria-lineage in Brazil, since its replacement with the Yamagata-lineage in the early 1990s (Hemphill et al., 1993). Antigenic results of representative isolates from 2001 and 2002, as well as genetic analysis of fourteen HA nucleotide sequences from 1999, 2001 and 2002 epidemics indicate that Brazilian influenza B viruses up to 2001 had been related to Yamagata-lineage, and those detected in 2002 associated with the Victoria lineage. All 2002 samples analyzed were closely related to B/NewYork/1/02, and lacked antigenic cross-reactivity with the influenza B vaccine strain used in Brazil throughout this epidemic.

## 2. Materials and methods

### 2.1. Rapid detection of viruses

Nasopharyngeal aspirates or nasal swabs were collected from subjects with influenza-like disease, from June to October throughout the 1999–2002 influenza epidemics in sentinel health units located in four Brazilian States: Rio Grande do Sul (RS), Santa Catarina (SC), Rio de Janeiro (RJ) and Espírito Santo (ES). Preliminary detection of common respiratory viruses (influenza A and B, RSV, adenovirus, parainfluenza 1, 2, and 3) in clinical samples was performed by IFA, in laboratories located in each State unit, using the Respiratory panel 1 Viral Screening and Identification kit (Chemicon International, CA, USA).

### 2.2. Virus isolation

IFA positive samples from ES, SC and RS States were sent in dry ice to Respiratory Viruses Laboratory (RVL) for isolation in Madin–Darby canine kidney (MDCK) tissue culture. Rio de Janeiro State samples were received in RVL immediately after collection in standard transportation medium at 4°C. These samples were inoculated into cell monolayers grown in 25 $cm^2$ cell culture flasks or in 6 well cell culture clusters (TPP, Switzerland) as described elsewhere (Kendal et al., 1982).

### 2.3. Antigenic analysis

Hemagglutination inhibition (HI) testing of 2001 and 2002 samples were performed using the WHO Influenza Reagent Kit for Identification of Influenza Isolates, produced and distributed by WHO Collaborating Center For Surveillance, Epidemiology and Control of Influenza in American Continent (CDC, USA) as described in the kit's manual. Distinct influenza B anti-sera were used for samples from subsequent epidemics: B/Beijing/184/93 (Yamagata-lineage) and B/Beijing/243/97 (Victoria-lineage) anti-sera for samples isolated in 2001, and B/Sichuan/379/99 (Yamagata-lineage) and B/Hong Kong/330/01 (Victoria-lineage) anti-sera, for 2002 isolates.

### 2.4. RNA extraction and cDNA synthesis

Total RNA was extracted directly from 150 μL of clinical specimen supernatant from IFA positive samples. The extraction was carried out using guanidinium thiocyanate method (Boom et al., 1990) and the RNA was eluted in 30 μL of RNAse-free Milli-Q water. Reverse transcription (RT) was performed with random hexamer primers as reported elsewhere (Ellis et al., 1995), except for the use of Avian Myeloblastosis Virus Reverse Transcriptase (2.5U) (Invitrogen, USA).

### 2.5. Multiplex PCR characterization and sequencing analysis

The resultant cDNA was used in a Multiplex PCR protocol for quick confirmation of influenza B samples, which were IFA positive. Sequencing was carried out using a Nested-PCR protocol generating overlapping fragments in the second PCR round. The Nested-PCR protocols for Multiplex and HA gene sequencing are described elsewhere (Ellis et al., 1995; Stockton et al., 1998). PCR amplicons used in sequencing reactions were purified from 1.8% agarose gels using QIAquick spin columns (Qiagen, Canada) as described in the manufacturer's manual and sequenced in an ABI 3100 capillary automatic sequencer using the Dye Deoxy Terminator method (Cycle Sequencing Ready Reaction Kit-version 3.1, Applied Biosystems, USA). Sequences of primers used for sequencing reactions are described by Ellis et al. (1995).

### 2.6. Genetic analysis

Sequences obtained in this study and others available in the Los Alamos Influenza Sequence Database (http://www.flu.lanl.gov) were aligned using ClustalW software (http://www.ebi.ac.uk/clustal) and edited using BioEdit Software, version 5.0.9 (Hall, 1999). Phylogenetic analysis was performed using Neighbor-Joining (NJ) algorithm, (MEGA software version 2.1 (Kumar et al., 2001)). In order to evaluate the robustness of the trees, the probabilities of the internal branches were determined through 1000 bootstrap replications. The list of accession numbers of HA gene of 2001 and 2002 samples sequenced in this study are shown in Tables 1 and 2, respectively.