Influenza B Viruses in the Population of Province of Vojvodina during the 2012/2013 Season: Differentiation of B/Yamagata and B/Victoria Lineages by Real-time RT-PCR, Antigenic and Phylogenetic Characterization

Jelena Radovanov[1], Vesna Milošević[1,2], Ivana Hrnjaković Cvjetković[1,2], Miljub Ristić[1,2], Milan Djilas[1], Nataša Nikolić[1], Aleksandra Patić[1,2], Gordana Kovačević[1], Aleksandra Jovanović Galović[1], Tamaš Petrović[3], Sandra Stefan Mikić[2,4]

[1]Institute of Public Health of Vojvodina, Novi Sad, Serbia;
[2]University of Novi Sad, Medical Faculty, Novi Sad, Serbia;
[3]Scientific Veterinary Institute Novi Sad, Novi Sad, Serbia;
[4]Clinic for Infectious Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia

Introduction At present, two phylogenetically distinct influenza B virus lineages, B/Yamagata and B/Victoria, co-circulate worldwide and can cause significant morbidity and mortality.
Objective The aim of this study was to determine the prevalences of two influenza B virus lineages in the population of Vojvodina and to identify their antigenic and phylogenetic properties.
Methods A total of 369 and 334 nasopharyngeal, or nasal/throat swab samples, collected during the 2012/2013 and 2013/2014 seasons, respectively, were tested using specific singleplex influenza A, influenza B, influenza B/Yamagata and influenza B/Victoria real-time reverse transcription polymerase chain reaction (RT-PCR) assays. Antigenic and genetic testing were done by hemagglutination inhibition assay and hemagglutinin and neuraminidase gene sequence analysis, respectively.
Results During the 2012/2013 season, influenza B viruses were present in 53.4% (101/189) of influenza positive samples. The B/Yamagata-like viruses (81.2%) significantly predominated over the B/Victoria-like viruses (18.8%). Comparing to B/Victoria-like positive patients, among B/Yamagata-like positive patients, children 5–14 years of age were significantly more represented (5.3% vs. 35.4%, respectively), as well as patients with mild form of illness (15.8% vs. 45.1%, respectively). The results of sequence analysis and antigenic testing showed that tested viruses were not closely related to B/Wisconsin/1/2010, the vaccine virus for 2012/2013. During the 2013/2014 season influenza B viruses were not detected.
Conclusion The results of this study confirmed the health significance of influenza B viruses and indicated that B/Yamagata-like viruses were significantly more prevalent than B/Victoria lineage viruses, during the 2012/2013 season. They also showed a sub-optimal match between the tested viruses and the vaccine virus for season 2012/2013.
Keywords: influenza B; acute respiratory infections; epidemic; real-time RT-PCR

INTRODUCTION

Influenza B virus is the only species in the genus *Influenzavirus B* belonging to the family Orthomyxoviridae. Together with members of genus *Influenzavirus A*, influenza B viruses cause influenza (flu), a highly contagious acute respiratory illness (ARI). Usually, it is mild, self-limited disease, but elderly persons, infants and patients with chronic medical conditions may develop a life-threatening severe acute respiratory illness (SARI) which demands hospitalization and/or treatment in an intensive care unit [1]. In some cases SARI progresses in the most severe form of acute lung injury named acute respiratory distress syndrome (ARDS) [1]. Although influenza A viruses are considered to represent the greatest public health concern, influenza B virus infections are also a significant cause of morbidity and mortality worldwide, with particularly severe outcomes in children and young adults [2, 3]. Influenza-related hospitalizations and complications such as myositis are more common in children infected with influenza B than in children with influenza A viruses [4].

In temperate climates, influenza is a seasonal epidemic disease occurring during the winter months. Epidemics are the consequence of "antigenic drift", small, ongoing changes in the amino-acid sequences of the antigenic portions of viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Those antigenic changes result from the accumulation of point mutations during viral replication, because of the lack of proofreading ability of viral RNA-dependent RNA polymerase [5]. Antigenic drift leads to antigenically new virus strain which can evade immunity to the previous strain. Influenza B viruses undergo antigenic drift less rapidly than influenza A viruses. Their HA has overall three to five times slower amino acid mutation rate, although the exact mechanism for such a slower mutation

Correspondence to:
Jelena RADOVANOV
Institute of Public Health of Vojvodina
Centre of Virology
Futoška St. 121, 21000 Novi Sad
Serbia
virusologija@izjzv.org.rs

rate remains unknown [6]. However, that characteristic is probably the major reason why they are associated with less frequent and less severe epidemics than influenza A viruses [7]. Also, influenza B viruses do not cause pandemics. Lack of pandemic potential of influenza B viruses is a consequence of the limited host range of the virus. Unlike influenza A viruses, influenza B viruses have evolved almost exclusively as human pathogens, which limits the generation of new strains by reassortment [8]. After the pandemic of influenza A(H1N1)pdm09 in 2009 and two subsequent seasons during which influenza A viruses predominated, the season 2012/2013 was the first season during which the substantial activity of influenza B viruses was recorded in most European countries (http://www.who.int/influenza/vaccines/virus/recommendations/en/).

Originally, influenza B viruses were a homogenous group which started to diverge into two genetically and antigenically distinguishable lineages in the late 1970s [4]. These two lineages with drift variants have circulated or co-circulated during different time periods in different geographic regions [9]. In the 1990s, B/Yamagata-like viruses became the dominant influenza B viruses, while B/Victoria lineage was mainly restricted to East Asia. However, during the 2000/2001 and 2001/2002 seasons, the B/Victoria lineage viruses re-emerged in North America and Europe and spread globally. At present, two phylogenetically and antigenically distinct lineages if influenza B viruses, the Yamagata lineage represented by the B/Yamagata/16/88 strain and the Victoria lineage represented by the B/Victoria/2/87, co-circulate worldwide [9].

The distinct antigenic properties of two influenza B lineages allow their differentiation by hemagglutination inhibition (HI) testing using specific immune sera raised against homologous strains of either lineage [4]. Differentiation of two lineages can also be done by real-time reverse transcription polymerase chain reaction (RT-PCR) which is a fast, specific, and sensitive diagnostic method that allows large-scale diagnostics.

Annual influenza vaccination is the most effective method for preventing influenza and associated complications. In general, seasonal influenza vaccines are trivalent, containing a mixture of strains of the circulating epidemic influenza A(H1N1)pdm09, A(H3N2) and B virus. A flu vaccine can protect against influenza viruses that are the same or related to the viruses in the vaccine. Influenza viruses evolve rapidly, so antigenic mutants are likely to emerge. World Health Organization (WHO) performs global monitoring of influenza viruses evolution and spread, using virological and epidemiological data collected by National Influenza Centers (NICs) through Global Influenza Surveillance and Response System (GISRS). WHO reviews the world epidemiological situation twice annually (in February for Northern Hemisphere and in September for Southern Hemisphere), and recommends the content of the influenza vaccine for the forthcoming influenza season. However, selecting the right influenza B virus strain has been proven very difficult. For instance, in the period 2001–2010, in the USA, influenza B virus strain in the vaccine missed the dominant influenza B virus lineage in circulation in five seasons [10]. Antibodies produced against two distinct lineages had limited cross-protection, so the result of mismatch was suboptimal immune response to vaccination, which resulted in a high frequency of illness and severe outcomes in all age groups [6]. To address this problem, WHO suggested in 2012 the addition of influenza B virus strains of both lineages in the next season's influenza vaccine, thus turning the conventional trivalent vaccine into a quadrivalent vaccine [10]. The immunogenicity and the lack of meaningful immune interference between virus strains in quadrivalent vaccine were proven in adults and in children [2].

OBJECTIVE

The aim of this study was to determine the prevalences of two influenza B virus lineages, B/Yamagata and B/Victoria, among patients with acute respiratory infections from Vojvodina region, during the 2012/2013 and 2013/2014 winter seasons. Also, we intended to identify the antigenic and genetic properties of representative viruses and to determine the phylogenetic relationships between them and the reference viruses.

METHODS

Detection of influenza B viruses RNA

A total of 369 and 334 nasopharyngeal or nasal/throat swab samples were collected from patients with acute respiratory infections, during the 2012/2013 and 2013/2014 study periods (October–May), respectively. Samples were transferred to WHO NIC situated in the Centre of Virology in the Institute of Public Health of Vojvodina in Novi Sad.

Samples were processed in accordance with WHO Global Influenza Surveillance Network recommended protocols [7]. Viral RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Germany). Influenza A and B virus detection and determination of B/Yamagata-like and B/Victoria-like viruses was done by singleplex real-time RT-PCR assays. Reverse transcription and amplification was performed using one-step AgPath-ID™ One-Step RT-PCR Reagents (Applied Biosystems, USA), and oligonucleotide primer and probe sets designed for detection of influenza A and B virus hemagglutinin gene segments, and influenza B lineage-specific sets of primers and probes, provided by Centers for Disease Control and Prevention (CDC, USA). Testing was done according to the CDC's instructions enclosed with the reagents. In brief, the master mix containing appropriate primers and probe was dispensed into reaction tube strips in a volume of 20 μl, and 5 μl of RNA extracts were added to each tube. Negative and positive template controls for all primer/probe sets were included in each run. The human RNase P gene primer and probe set served as an internal positive control for human RNA. Real-time RT-PCR was performed on Applied Biosystems 7500 (Applied Biosystems, USA)

doi: 10.2298/SARH1508429R

real-time thermocycler. Thermocycling real-time RT-PCR conditions were as follows: reverse transcription for 30 minutes at 50°C, Taq-polymerase activation for 10 minutes at 95°C, and 45 cycles of denaturation at 95°C for 15 seconds and extension at 55°C for 30 seconds. Results were analyzed using Applied Biosystems 7500 Software version 2.0.6 and the interpretation of data was done according to WHO guidelines [7].

Antigenic and phylogenetic analysis

Four influenza B positive samples were sent to the WHO Collaborating Centre for Reference and Research on Influenza (CCRRI) in National Institute for Medical Research, (London, UK) for antigenic testing of viruses by HI assay. Two of them were subjected to genetic characterization of viruses by hemagglutinin and neuraminidase gene sequence analysis.

Statistical analysis

Comparisons of influenza B lineages distribution by age, clinical manifestation of illness and underlying/medical conditions were done by Fisher's exact test and binomial sign test. A p-value <0.05 was considered statistically significant.

RESULTS

During the 2012/2013 winter season, in the Vojvodina region, influenza activity increased through January and February before peaking in late February. Out of 369 samples tested by real-time RT-PCR, influenza viruses were detected in 50% (189/369). Among positive samples there were 101 (53.4%) influenza B positive, and 88 (46.6%) influenza A positive. The results of real-time RT-PCR testing are summarized in Table 1. Of the influenza B positive samples,

Table 1. Distribution of B/Yamagata-like and B/Victoria-like viruses by age, clinical manifestation of illness and chronic medical/underlying condition

Variable		B/Yamagata-like virus positive patients		B/Victoria-like virus positive patients		p-value[a]
		N	%	N	%	
Age (years)	0–4	9	11.0	2	10.5	>0.05
	5–14	29	35.4	1	5.3	0.0106
	15–29	3	3.6	2	10.5	>0.05
	30–64	30	36.6	11	57.9	>0.05
	≥65	11	13.4	3	15.8	>0.05
	Total	82	81.2	19	18.8	<0.0001
Clinical manifestation	ARI	37	45.1	3	15.8	0.0203
	SARI	39	47.6	12	63.2	>0.05
	ARDS	6	7.3	4	21.0	>0.05
	ARDS with fatal outcome	3	3.7	1	5.3	>0.05
Chronic medical/ underlying condition	Patients with reported risk factor	33	40.2	12	63.2	>0.05
	One risk factor	21	63.6	7	58.3	
	More than one risk factor	12	36.4	5	41.7	
Risk factors[b]	Chronic cardiovascular disease	8	24.2	4	33.3	>0.05
	Chronic respiratory disease	7	21.2	1	8.3	
	Diabetes	8	24.2	3	25.0	
	Malignancy	7	21.2	4	33.3	
	Immunodeficiency	9	27.3	4	33.3	
	Chronic liver/renal disease	6	18.2	0	0	
	Morbid obesity[c]	3	9.1	0	0	
	Pregnancy	0	0	1	8.3	

[a] P-values were calculated using Fisher's exact test, except for total number of B/Yamagata-like and B/Victoria-like virus positive patients, where binomial sign test was used.
[b] Patients with multiple risk factors were counted for each.
[c] Defined as body mass index of or greater than 40.

N – number of patients; ARI – acute respiratory illness; SARI – severe acute respiratory illness; ARDS – acute respiratory distress syndrome with or without fatal outcome

Table 2. Association of clinical manifestation of influenza B virus infections with chronic medical/underlying conditions

Chronic medical/underlying condition	Clinical manifestation of illness								Total
	ARI		SARI		ARDS		ARDS with fatal outcome		
	N	%	N	%	N	%	N	%	
Without chronic medical/underlying condition	35	62.5	19	33.9	2	3.6	0	0	56
With chronic medical/underlying condition	5	11.1	32	71.1	8	17.8	4	8.9	45
p-value*	<0.0001		0.0003		0.0219		0.0365		

* P-values were calculated using Fisher's exact test.

B/Yamagata-like viruses were detected in 81.2% (82/101) and B/Victoria-like viruses in 18.8% (19/101), which was a highly significant difference (p<0.0001). Comparing to B/Victoria-like positive patients, among B/Yamagata-like positive patients, children 5–14 years of age were significantly more prevalent (5.3%, 1/19 vs. 35.4%, 29/82, respectively, p=0.0106), as were the patients with mild form of illness – ARI (15.8%, 3/19 vs. 45.1%, 37/82, respectively, p=0.0203). We found no significant differences in distribution of influenza B virus lineages according to the presence/absence or type of any chronic medical/underlying condition. However, the presence of a chronic medical/underlying condition was a significant risk factor for developing the severe forms of disease – SARI (71.1%, 32/45 patients with, vs. 33.9%, 19/56 patients without any chronic medical/underlying condition, p=0.0003) and ARDS (17.8%, 8/45 patients with, vs. 3.6%, 2/56 patients without a chronic medical/underlying condition, p=0.0219), as well as for the fatal outcome of illness (8.9%, 4/45 patients with, vs. none of the patients without a chronic medical/underlying condition, p=0.0365) (Table 2). The fatal outcome of influenza was detected in one patient with chronic renal disease, in one patient with malignancy, and in two morbidly obese patients. In the group of patients without recorded preexisting risk conditions, the mild form of illness (ARI) was significantly more common (62.5%, 35/56) than in patients with chronic medical/underlying condition (11.1%, 5/45, p<0.0001).

The sequence analysis of the HA (Figure 1) gene and the NA (Figure 2) gene was carried out on two of the influenza B/Yamagata virus isolates, B/Serbia/NS219/2013 and B/Serbia/NS249/2013. Both sequenced viruses were in clade 2 and showed little phylogenetic distinction. They were more closely related to 2013/2014 vaccine virus B/Massachusetts/2/2012, and to some viruses from Russia, Ukraine, Hong-Kong, Romania, Greece and Italy, than to viruses from other parts of Serbia – B/Belgrade/1481/2013, B/Leskovac/905/2013 and B/Cupria/1097/2013.

In HI, viruses from each of four positive samples showed a very similar pattern of reactivity with the panel of postinfection ferret sera and one hyperimmune sheep serum (Table 3). It was clear that test viruses carrying clade 2 HA genes could be differentiated antigenically by certain antisera. In general, test viruses displayed better reactivity with antisera raised against viruses belonging to genetic clade 2, compared with antisera raised against viruses from genetic clade 3. Antiserum against B/Wisconsin/1/2010, the vaccine virus for 2012/2013 belonging to genetic clade 3, only recognized the test viruses with a titre four-fold reduced compared with its titre to the homologous antigen. Antiserum raised against other clade 3 virus, B/Novosibirsk/1/2012, failed to recognize any of the test viruses with a titre within four-fold of its recognition of the homologous virus. An antiserum against B/Stockholm/12/2011, another clade 3 virus, recognized the test viruses somewhat better – all test viruses were recognized by the antiserum at a titre within four-fold of the titre of the antiserum to the homologous antigen.

Antisera raised against B/Massachusetts/2/2012, belonging to genetic clade 2, propagated in MDCK (Madin–Darby Canine Kidney) cell cultures or in embryonated chicken eggs, as well as antisera against B/Estonia/55669/2011 and B/Hong Kong/3577/2012, recognized all the viruses well reacting with a titre within two-fold of their titres of their homologous viruses. However, antiserum against B/Brisbane/3/2007, the vaccine virus for 2011/2012 failed to inhibit the hemagglutination of test viruses with a titre within four-fold of its titre to the homologous virus. The same result was observed with postinfection ferret sera against B/Florida/4/2006, the vaccine virus for 2008/2009, while hyperimmune sheep serum against the same reference virus recognized all test viruses with a titre four-fold reduced compared with its titre to the homologous antigen.

During the 2013/2014 winter season, a total of 334 nasopharyngeal or nasal/throat swab samples were tested by real-time RT PCR. Influenza B virus were not identified in any sample, while influenza A viruses were detected in 34.7% (166/334) of them.

DISCUSSION

During the 2012/2013 winter season, in the European Region, influenza activity was greater than that seen in previous season, although the relative proportions of type A and B varied between the countries [11]. According to data collected from NICs situated in European countries, the overall proportion of influenza B positive samples was 30% (9029/30133), which is significantly higher compared to 9.7% (4407/45236) recorded in season 2011/2012 [12]. In some countries, like England, Scotland, Spain, France and Switzerland, influenza B viruses were predominant or both types of influenza viruses were equally represented. In accordance with that, in our province, infections with influenza B type were detected in more than half (53.4%, 101/189) of positive patients. The high proportion of influenza B cases recorded in 2012/2013 season might be explained by a substantial level of preexisting population immunity to influenza A viruses, acquired during several previous seasons dominated by the influenza A viruses. Namely, according to data available from FluNet (http://www.who.int/influenza/gisrs_laboratory/flunet/en/), a global tool for influenza virological surveillance established by GISRS, since the pandemic season 2009/2010 until the season 2012/2013, influenza B viruses were detected only sporadically in Serbia. Data from FluNet indicated that during the season 2012/2013, influenza B virus infections accounted for about half of influenza positive cases from Serbia. Virological surveillance was continued during the 2013/2014 season in Vojvodina, but no influenza B viruses were detected in any of the samples tested, while 34.7% (166/334) of them were influenza A virus positive. No influenza B positive cases from Serbia were reported to FluNet. Low activity of influenza B viruses was seen in most of European countries. According to data reported to the European Centre for Disease Prevention and Control (ECDC) until week 07/2014, among 4,217 influenza virus positive sentinel specimens and 10,788 influenza positive

doi: 10.2298/SARH1508429R

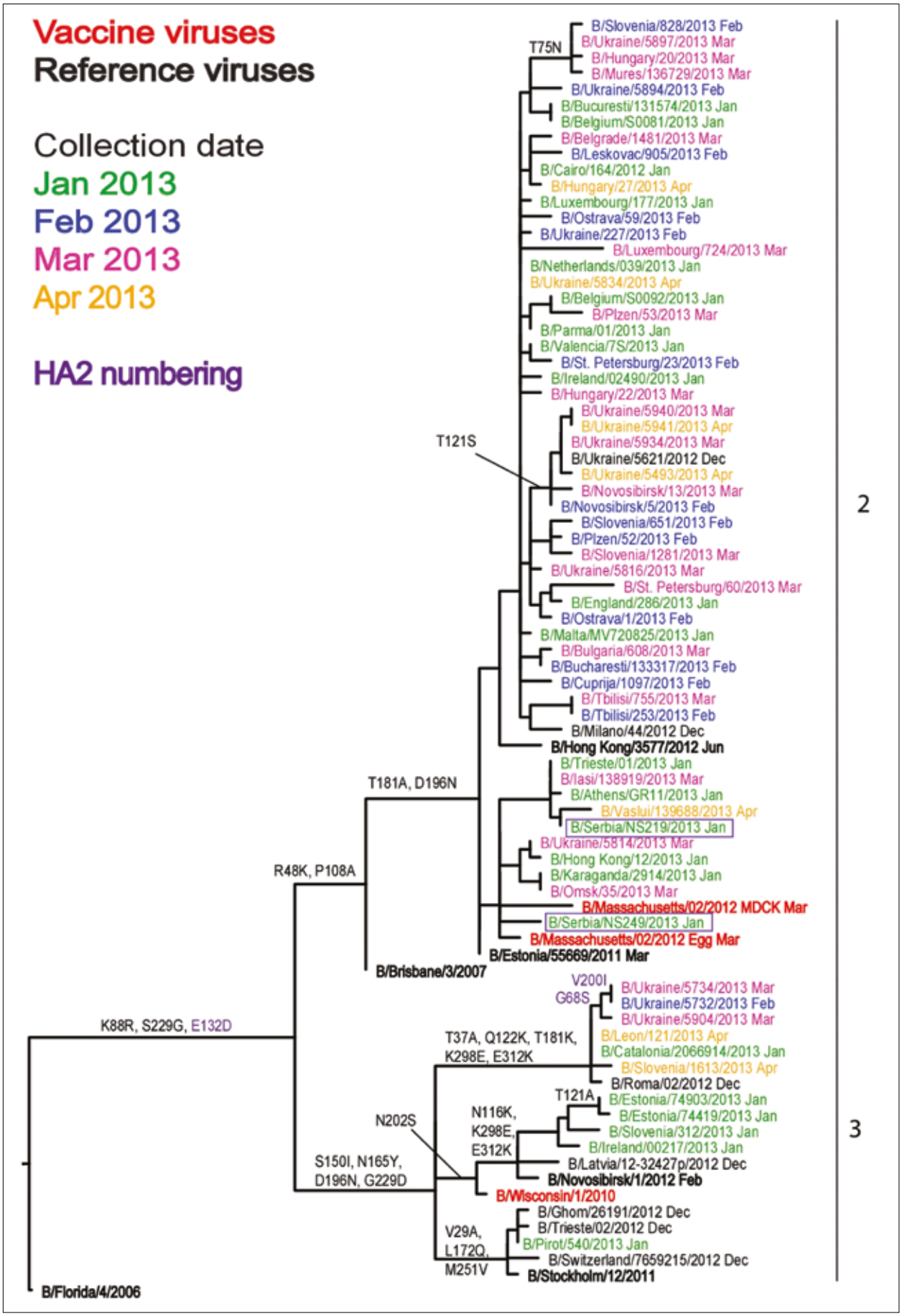

Figure 1. Phylogenetic comparison of influenza B (Yamagata-lineage) HA genes (courtesy of John McCauley, WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research, London, UK)

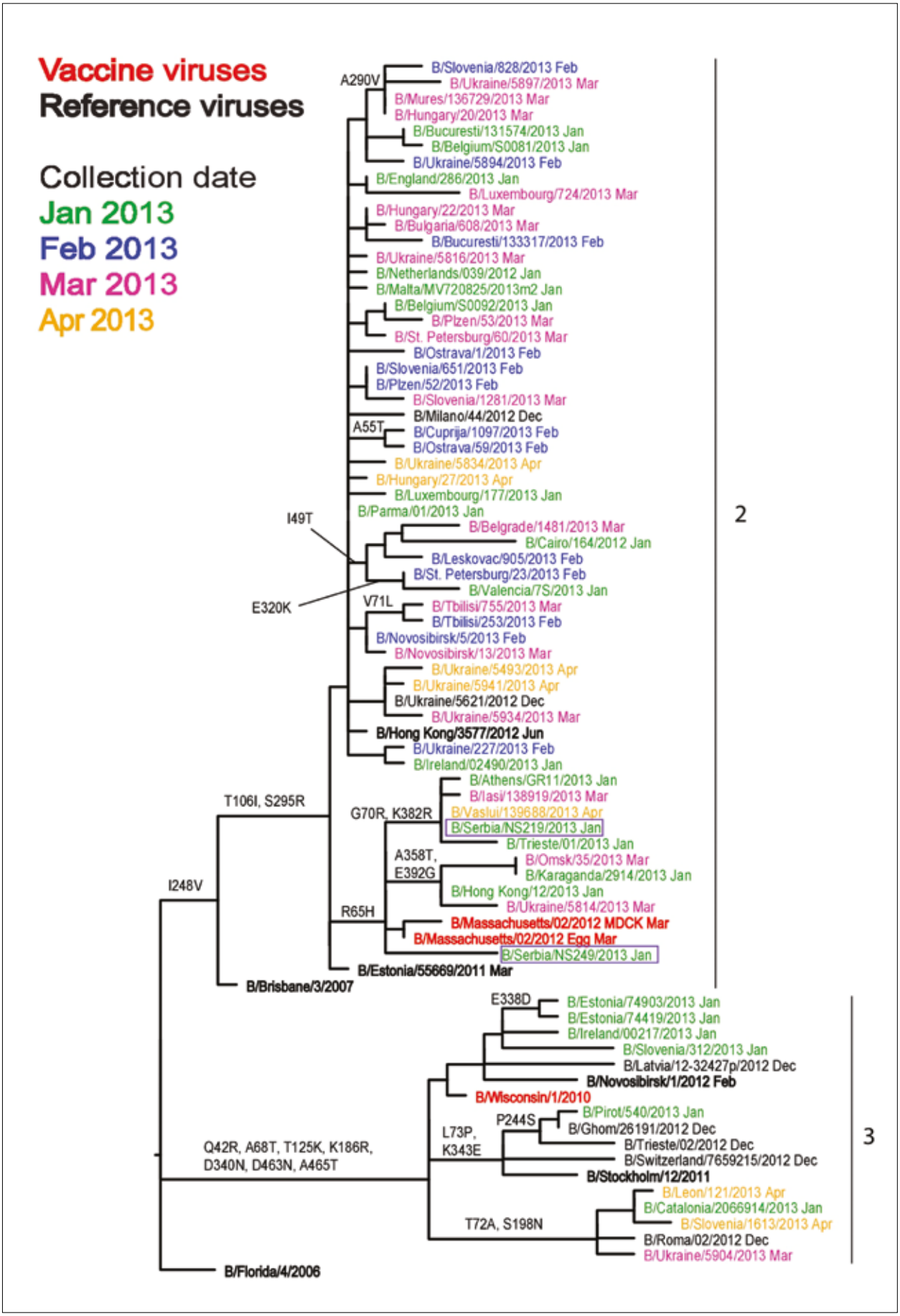

Figure 2. Phylogenetic comparison of influenza B (Yamagata-lineage) NA genes (courtesy of John McCauley, WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research, London, UK)

doi: 10.2298/SARH1508429R

Table 3. Antigenic analyses of influenza B viruses by hemagglutination inhibition assay (courtesy of John McCauley, WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research, London, UK)

Viruses		Collection date	Passage history	Hemagglutination inhibition titre									
				Postinfection ferret sera									
				B/Fl	B/Fl[a]	B/Bris	B/Wis	B/Stock	B/Estonia	B/Novo	B/HK	B/Mass	B/Mass
				4/06	4/06	3/07	1/10	12/11	55669/11	1/12	3577/12	2/12	2/12
				SH479	F1/10	F21/12	F24/12	F12/12	F26/11	F31/12	F33/12	Egg F02/13[b]	T/C F03/13
					Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 2	Group 2	Group 2
Reference viruses	B/Florida/4/2006	2006-12-15	Egg[b]	5120	640	640	320	320	160	40	320	1280	80
	B/Brisbane/3/2007	2007-09-03	Egg[b]	5120	640	640	320	640	320	40	320	1280	160
	B/Wisconsin/1/2010	2007-08-07	Egg[b]	1280	320	320	320	320	10	80	80	320	40
	B/Stockholm/12/2011	2007-08-07	Egg[b]	1280	160	160	160	160	<40	40	40	320	20
	B/Estonia/55669/2011	2011-03-14	MDCK[c]	640	80	80	40	40	640	80	640	160	160
	B/Novosibirsk/1/2012	2012-02-14	MDCK[c]	2560	160	160	160	320	320	320	320	320	320
	B/Hong Kong/3577/2012	2012-06-13	MDCK[c]	2560	160	160	80	160	1280	160	640	320	640
	B/Massachusetts/02/2012	2012-03-13	Egg[b]	640	160	80	40	40	160	20	320	160	160
	B/Massachusetts/02/2012	2012-03-13	MDCK[3]	2560	320	640	160	320	160	40	320	640	160
Test viruses	B/Serbia/NS-196/2013	2013-01-08	MDCK[c]	1280	80	80	80	40	320	40	320	160	160
	B/Serbia/NS-217/2013	2013-01-21	MDCK[c]	1280	80	80	80	80	320	40	640	160	160
	B/Serbia/NS-219/2013	2013-01-23	MDCK[c]	1280	80	80	80	40	320	40	320	160	80
	B/Serbia/NS-249/2013	2013-01-31	MDCK[c]	1280	80	80	80	40	640	40	320	160	160

[a] Hyperimmune sheep serum
[b] Embryonated chicken egg
[c] Madin–Darby canine kidney cell culture

specimens collected for diagnostic purposes in hospitals, influenza B viruses were found in only 2% and 3% of them, respectively [13].

In the 2010/2011 season B/Victoria-like viruses predominated globally, except in China, where B/Yamagata-like viruses were most commonly detected [14]. Next season, both lineages were observed in similar proportions in many countries, suggesting an increase in the prevalence of viruses of the B/Yamagata lineage [15]. From September 2012 to January 2013, influenza B viruses of the Victoria and the Yamagata lineages co-circulated. Although B/Victoria lineage was prevalent in some countries, B/Yamagata-like viruses have continued to increase in proportion and between February and September 2013 became dominant in all countries reporting influenza B infections [11, 16]. The results of this study indicate that, in 2012/2013 season, in Vojvodina region, B/Yamagata lineage viruses also predominated over those of the B/Victoria lineage. Among the B/Yamagata-like positive patients there were significantly more children 5–14 years old and patients with mild form of illness. Literature data concerning the distribution of influenza B virus lineages by age or clinical manifestations of illness are contradictory. Chi et al. [17] found that the B/Yamagata-like strains were associated with more invasive infections of lower respiratory tract, while Tan et al. [9] detected no significant difference in clinical severity by influenza B lineage, but found that people infected by the B/Yamagata lineage were older than those infected by the B/Victoria lineage. Although the results of this study did not associate influenza B lineages with distinct chronic diseases or conditions, they confirmed the importance of chronic medical/underlying conditions as a risk factors for developing severe forms of influenza B virus infections and influenza-related deaths. Analysis of nucleotide sequence of the HA gene revealed that both influenza B/Yamagata viruses were in phylogenetic clade 2, the most commonly seen clade in recent months. According to WHO CCRRI [12], the HA genes of most B/Yamagata lineage viruses from 2012/2013 season fell within genetic clade 2 or 3, with the proportion of viruses in clade 2 markedly increasing in many areas during this period. Although the tested viruses from Vojvodina and viruses from other parts of Serbia fell into a single phylogenetic clade, phylogenetic three indicated co-circulation of genetically different strains of influenza B viruses in our country, during the single epidemic season. Viruses in clade 2 are generally antigenically distinguishable from those in clade 3, as can be seen with the results of HI with some post-infection ferret antisera.

The antigenic distance between the vaccine candidate and the circulating strain of influenza virus can be estimated by HI. Four-fold change of HI titer of the circulating strain in the presence of antisera to the vaccine candidate is considered to be a substantial antigenic difference and suggests a sub-optimal match between vaccine candidate and circulating strain [18]. Apart from preexisting population immunity to influenza A viruses, the high proportion of influenza B cases recorded in 2012/2013 season can partially be explained by a mismatch of the influenza B vaccine and the circulating strain. Namely, in this study, antiserum against B/Wisconsin/1/2010, the vaccine virus for 2012/2013 recognized the test viruses with a titre four-fold reduced compared with its titre to the homologous antigen, while antisera raised against B/Massachu-

setts/2/2012, recognized all the viruses well reacting with a titre within two-fold of their titres of their homologous viruses. The results of sequencing showed that B/Massachusetts/2/2012 and test viruses belong to the same phylogenetic clade 2 and that they were more distantly related to B/Wisconsin/1/2010 and other viruses from clade 3. These findings were in correlation with the results of antigenic analysis of most recent B/Yamagata-like influenza B viruses. Most of them were antigenically distinguishable from the 2012/2013 vaccine virus B/Wisconsin/1/2010 and were more closely related to B/Massachusetts/2/2012-like viruses [12]. As a result of this finding, WHO recommended the change of influenza B virus component of the vaccine intended for use in the 2013/2014 influenza season [11]. Previous vaccine strain B/Wisconsin/1/2010 was replaced with B/Massachusetts/2/2012-like virus belonging to Yamagata lineage, while quadrivalent vaccines also contain additional B/Brisbane/60/2008-like virus belonging to B/Victoria lineage.

CONCLUSION

According to our knowledge, this was the first investigation of prevalence of different influenza B virus lineages in our population. The results of this study revealed that influenza B/Yamagata-like viruses significantly predominated over the B/Victoria-like viruses and that among the B/Yamagata-like positive patients there were significantly more children 5–14 years old and patients with mild form of the illness. The presence of two influenza B virus lineages was not associated with a distinct chronic disease or condition. Among patients with chronic medical/underlying conditions, the proportions of severe forms of influenza B virus infections, SARI and ARDS, and fatal cases, were significantly higher than among patients with no chronic conditions. The tested viruses were phylogenetically more closely related and antigenically more similar to 2013/2014 vaccine virus B/Massachusetts/2/2012 then to 2012/2013 vaccine virus B/Wisconsin/1/2010. A long-term research study is needed for better understanding of the complex epidemiological dynamics of influenza B virus lineages in population of Province of Vojvodina.

ACKNOWLEDGEMENTS

The presented work is part of the research done in the project TR31084 granted by the Serbian Ministry of Education, Science and Technological Development.

We are very grateful to John McCauley and colleagues from the WHO Collaborating Centre for Reference and Research on Influenza in National Institute for Medical Research (London, UK) for the support and collaboration.

REFERENCES

1. Petrović V, Šeguljev Z, Ćosić G, Ristić M, Nedeljković J, Dragnić N, et al. Overview of the winter wave of 2009 pandemic influenza A(H1N1)v in Vojvodina, Serbia. Croat Med J. 2011; 52(2):141-50.
2. Toback SL, Levin MJ, Block SL, Belshe RB, Ambrose CS, Falloon J. Quadrivalent Ann Arbor strain live-attenuated influenza vaccine. Expert Rev Vaccines. 2012; 11(11):1293-303.
3. Dolin R. The quadrivalent approach to influenza vaccination. J Infect Dis. 2013; 208(4):539-40.
4. Biere B, Bauer B, Schweiger B. Differentiation of influenza B virus lineages Yamagata and Victoria by real-time PCR. J Clin Microbiol. 2010; 48(4):1425-7.
5. Taubenberger JK, Morens DM. The pathology of influenza virus infections. Annu Rev Pathol. 2008; 3:499-522.
6. Wang Q. Influenza type B virus haemagglutinin: antigenicity, receptor binding and membrane fusion. In: Wang Q, Tao YJ, editors. Influenza Molecular Virology. Norfolk: Caister Academic Press; 2010. p.29-52.
7. World Health Organization Global Influenza Surveillance Network – WHO GISN. Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza. Geneva, Switzerland: WHO Press; 2011.
8. Hampson AW, Mackenzie JS. The influenza viruses. Med J Aust. 2006; 185(10):S39-43.
9. Tan Y, Guan W, Lam TTY, Pan S, Wu S, Zhan Y, et al. Differing epidemiological dynamics of influenza B lineages in Guangzhou, southern China, 2009-2010. J Virol. 2013; 87(22):12447-56.
10. Domachowske JB, Pankow-Culot H, Bautista M, Feng Y, Claeys C, Peeters M, et al. A randomized trial of candidate inactivated quadrivalent influenza vaccine versus trivalent influenza vaccines in children aged 3-17 years. J Infect Dis. 2013; 207(12):1878-87.
11. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2013-14 northern hemisphere influenza season. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/2013_14_north/en/ [accessed 2014 May 15].
12. World Health Organization Collaborating Centre for Reference and Research on Influenza, London – WHO CCRRI. Report prepared for the WHO annual consultation on the composition of influenza vaccine for the Northern Hemisphere 2013/14. Available from: http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_February_2013.pdf [accessed 2014 May 15].
13. ECDC. Seasonal influenza 2013–2014 in the EU/EEA countries. European Centre for Disease Prevention and Control. Available from: http://www.ecdc.europa.eu/en/publications/publications/influenza%20risk%20assessment%202014%20final.pdf [accessed 2014 May 10].
14. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2011-12 northern hemisphere influenza season. Available from: http://www.who.int/influenza/vaccines/2011_02_recommendation.pdf?ua=1 [accessed 2014 May 15].
15. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2012-13 northern hemisphere influenza season. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/201202_recommendation.pdf?ua=1 [accessed 2014 May 15].
16. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2013 southern hemisphere influenza season. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/201209_recommendation.pdf?ua=1 [accessed 2014 May 15].
17. Chi CY, Wang SM, Lin CC, Wang HC, Wang JR, Su IJ, et al. Clinical features of children infected with different strains of influenza B in southern Taiwan. Pediatr Infect Dis J. 2008; 27(7):640-5.
18. Tosh PK, Jacobson RM, Poland GA. Influenza vaccines: from surveillance through production of protection. Mayo Clin Proc. 2010; 85(3):257-73.

Вируси инфлуенце Б међу становништвом покрајине Војводине током сезоне 2012/2013: диференцијација линија *B/Yamagata* и *B/Victoria* помоћу тестова *real-time RT-PCR* и антигенска и филогенетска карактеризација

Јелена Радованов[1], Весна Милошевић[1,2], Ивана Хрњаковић Цвјетковић[1,2], Миољуб Ристић[1,2], Милан Ђилас[1], Наташа Николић[1], Александра Патић[1,2], Гордана Ковачевић[1], Александра Јовановић Галовић[1], Тамаш Петровић[3], Сандра Стефан Микић[2,4]

[1]Институт за јавно здравље Војводине, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[3]Научни институт за ветеринарство, Нови Сад, Србија;
[4]Клиника за инфективне болести, Клинички центар Војводине, Нови Сад, Србија

КРАТАК САДРЖАЈ

Увод Тренутно две филогенетски различите линије вируса инфлуенце Б, *B/Yamagata* и *B/Victoria*, заједно циркулишу широм света и могу изазвати значајан морбидитет и морталитет становништва.

Циљ рада Циљ рада је био да се одреди заступљеност две линије вируса инфлуенце Б међу становништвом покрајине Војводине и испитају антигенске и филогенетске особине репрезентативних вируса.

Методе рада Укупно 369 и 334 назофарингеална бриса или бриса носа и грла, сакупљена током сезоне 2012/2013, односно 2013/2014, анализирана су специфичним, појединачним инфлуенца А, инфлуенца Б, инфлуенца *B/Yamagata* и инфлуенца *B/Victoria real-time RT-PCR* тестовима. Антигенске и генетичке анализе вршене су реакцијом инхибиције хемаглутинације, односно анализом генске секвенце хемаглутинин и неураминидаза гена.

Резултати Током сезоне 2012/2013. вируси инфлуенце Б установљени су код 53,4% (101/189) инфлуенца-позитивних узорака. *B/Yamagata-like* вируси су били статистички значајно чешћи од *B/Victoria-like* вируса (81,2% наспрам 18,8%). У поређењу с пацијентима позитивним на *B/Victoria*, међу пацијентима позитивним на *B/Yamagata* било је статистички значајно више деце узраста 5–14 година (5,3% наспрам 35,4%) и болесника са благим обликом инфекције (15,8% наспрам 45,1%). Резултати секвенцирања и антигенског тестирања показали су да испитани вируси нису у блиској сродности са *B/Wisconsin/1/2010*, вакциналном вирусу за сезону 2012/2013. Током сезоне 2013/2014. вируси инфлуенце Б нису откривени.

Закључак Резултати овог истраживања су потврдили здравствени значај вируса инфлуенце Б и указали на то да су *B/Yamagata-like* вируси били статистички значајно чешћи у односу на вирусе линије *B/Victoria* у сезони 2012/2013. Они су такође показали субоптимално поклапање испитаних вируса и вакциналног вируса за сезону 2012/2013.

Кључне речи: инфлуенца Б; акутна респираторна инфекција; епидемија; *real-time RT-PCR*

Примљен • Received: 07/07/2014 **Прихваћен • Accepted:** 01/10/2014