SHORT COMMUNICATION

Influenza B in a temperate region of northern India 2010–2016: co-circulation of the two lineages with northern hemispherical seasonality

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Abstract Scant data exist about the epidemiology of influenza B in India. We set out to address the epidemiology of influenza B in a temperate region of northern India from 2010 to 2016. Outpatient and inpatient surveillance was conducted in patients presenting with acute respiratory infection in a northern Indian hospital from September 2010 till April 2016. After recording clinical data, combined nasal/throat swabs were collected and tested for influenza viruses by real time RT-PCR. Influenza A viruses were further subtyped into A/H3N2 and A/H1N1 whereas influenza B were differentiated into B/Yamagata and B/Victoria. Virus isolation, haemagglutination inhibition testing, sequencing and phylogenetic analysis was carried out on representative samples. Of the 6879 recruited cases, influenza B was detected in 299 (4.3%). The patients presented with respiratory symptoms of varying duration; cough, fever and nasal discharge being the most common. The peaking of the activity of the circulation showed a correlation with the onset of the winter with reduced temperatures and high dry humidity. B/Victoria lineage was detected in 35.4% (n = 106/299) whereas 53.8% (n = 161/299) were B/Yamagata. The circulation in each season was dominated by one lineage which correlated with the vaccine strain, but up to 37% consisted of a different lineage. We conclude that Influenza B exhibits a northern hemispherical seasonality in temperate northern

India with co-circulation of the 2 lineages of influenza B. These findings have relevance for vaccine effectiveness and argue for vaccination with a quadrivalent influenza vaccine.

Keywords Influenza · Influenza B virus · B/Yamagata · B/Victoria · Trivalent influenza vaccine · Quadrivalent influenza vaccine

Influenza viruses are a major cause of acute respiratory tract infection (ARI) with considerable morbidity and mortality. The influenza virus belongs to the family Orthomyxoviridae with a genome make-up of eight single-stranded, negative-sense RNA segments. Influenza A and B are the two types of influenza viruses that cause human disease. Influenza A viruses are further separated into subtypes and influenza B into lineages, based on their characteristics of their two surface antigens, hemagglutinin (HA) and neuraminidase (NA). Novel influenza virus variants emerge as a result of mutations and recombination events that occur during viral replication, resulting in frequent antigenic change [20].

Influenza B infections constitute between 20 and 30% of total influenza incidence but can be 50% or greater depending on the season [6]. Influenza B viruses have two main lineages, B/Yamagata and B/Victoria, that have co-circulated in most influenza seasons since the 1980s [5, 11, 15]. Contrary to influenza A virus, influenza B viruses undergo antigenic drift less rapidly [5]. While influenza A has a variety of subtypes as a result of its ability to infect a variety of vertebrate hosts, humans are the primary hosts for influenza B viruses [13]. The narrow host range is thought to limit subtype diversity and gene reassortment. Further, the rate of antigenic drift has been

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estimated to be slower for influenza B than for A viruses [13]. As a result, influenza B viruses evolve slower than influenza A viruses and there has been no evidence of influenza pandemic caused by a type B virus [7].

While surveillance has focussed on influenza A in the post-pandemic period in India, there is a general paucity of literature about the epidemiology of influenza B viruses. We have earlier reported influenza B viruses to be responsible for causing acute respiratory infections [9] and even an outbreak in an isolated nomadic community in the higher reaches of north India [8].

India is a large landmass with sub-regional variations in the seasonality of circulation of influenza viruses with northern temperate regions exhibiting a northern hemispherical seasonality whereas the rest of the country exhibiting a southern hemispherical seasonality, despite the geographical location of the whole country in the northern hemisphere [4, 10, 18]. Recently Saha et al. reported that circulation of influenza B may also be related to the latitude of the region in Asian countries [18]. We herewith report our experience with influenza B virus from 2010 to 2016 in Kashmir province of the northern state of Jammu and Kashmir, India that has a temperate climate with respiratory illnesses constituting the bulk of hospital visits during the winter months, either in the form of acute respiratory illnesses or as exacerbations of underlying chronic lung diseases. We performed surveillance for outpatients with influenza-like illness (ILI) and in-patients with severe acute respiratory illness (SARI) during the 2010–2011, 2011–2012, 2012–2013, 2013–2014, 2014–2015 and 2015–2016 influenza seasons at a 850-bedded tertiary care hospital. We defined ILI as fever of 100 °F (> 37.2 °C) accompanied by cough and/or sore throat, whereas SARI was defined as those patients with ILI who also require hospitalization. After recording history and examination, combined throat and nasal swabs were collected in viral transport medium, transported immediately to the influenza laboratory and tested by real-time RT-PCR for influenza viruses. From children < 5 years of age, nasopharyngeal swabs were collected whereas combined nasopharyngeal and oropharyngeal swabs were collected from patients > 5 years of age. All influenza A positive samples were further subtyped into A/H1N1pdm09 and A/H3 and Influenza B positive specimens distinguished into influenza B/Yamagata and Victoria lineages by real-time PCR using TaqMan probe. Representative type B positive specimens were sent to National Institute of virology for virus isolation and genetic characterization. Attempts were made to isolate the influenza virus using Madin-Darby Canine Kidney (MDCK) cells. The procedures used for MDCK cell maintenance and virus isolation were as per WHO manual and as described earlier [14].

Hemagglutination and hemagglutination inhibition (HI) tests were performed for antigenic characterization of the isolates using WHO influenza characterization Kit. To determine the hemagglutination titer of the isolates, two-fold serial dilutions of the viral sample in phosphate buffered saline (PBS)(50 µl volume) were mixed with 50 µl of 0.75% Guinea red blood cells (GRBCs) in PBS in 96-well plates. Following 45 min of incubation at 4 °C, wells exhibiting complete agglutination of GRBC were judged to be hemagglutination positive. Hemagglutination titer was defined as the reciprocal number of the hemagglutination positive viral dilution. For the HI test, twofold serial dilutions of antiserum in PBS (25 µl volume) provided were mixed with 25 µl of virus antigen adjusted to 8 hemagglutination units/50 µl. Following 60 min incubation at room temperature (20–25 °C), 50 µl of 0.75% GRBC in PBS was added to each well of the 96-well plates. Subsequent to further 45 min incubation at 4 °C, wells demonstrating inhibition of hemagglutination were judged as HI positive. HI values were defined as the reciprocal number of the antiserum dilution effective for HI.

Viral RNA was extracted from 11 isolates (4 isolates of 2012, 2 isolates of 2013 and 5 isolates of 2014) isolates using the Mag Max 96 Kit (Ambion). One step PCR (RT-PCR) (Invitrogen Superscript III Platinum kit) was used to amplify entire HA gene (~ 1700 bp) except one isolate of 2013 with HA1 (~ 1200 bp) and NA gene (~ 1400 bp) in three overlapping fragments of Type B using primer information provided by WHO CC Australia.

PCR amplicons were subjected to DNA sequencing using the Big Dye Terminator Kit Ver.3.1. The expected amplicons for each fragment were visualized on 2% agarose and purified using Charge switch magnetic beads PCR purification kit (Invitrogen). DNA sequencing was carried out using Big Dye terminator V 3.1 cycle sequencing ready reaction kit (ABI, Foster City, CA) and unincorporated labeled ddNTP's were purified using DyeEx 2.0 dye-terminated removal kit (Qiagen). The sequencing was done on ABI 3730 DNA analyzer. The obtained sequence information was edited by Seascap V2.5 software (Applied Biosystems) and pair wise sequence alignment and phylogeny was performed with MEGA 6 program. Phylogeny was constructed using Neighbor-joining (NJ) trees with Tamura-Nei Model best fit for Influenza. Sequence data were deposited with the gene bank database under accession numbers: *NA gene MH698952 to MH698962 and HA gene MH 703061- MH 703071*. Statistical analysis of all data was done using STATA 11 software. The study has been approved by the Institute Ethics Committee of SKIMS and informed consent for participation was obtained for all patients.

During the study period, 6879 samples were collected from patients with ARI/ILI/SARI aged 1 month–95 years

(median = 24 years, males = 54.7%). Influenza B was confirmed in 299 (4.3%) patients (Fig. 1). The age-wise positivity for influenza B varied from 2.7 to 9.4% being 2.7% (55/2062) in the < 5 years age, 9.4% (43/459) in the 5–18 years age group, 6.4% (174/2721) in the 18–60 years age group and 4.7% (27/578) in the > 60 years age group. The patients presented with various respiratory symptoms of 1–7 days (median = 2 days) (Table 1). Symptoms of fever, cough, chills/rigors, breathlessness, body-ache and headache were seen significantly more frequently in influenza B patients compared to those who were negative for influenza. A history of ARI in the family was elicited in 24.1%.

The seasonal monthly positivity is depicted in Fig. 1. Influenza B co-circulated with influenza A viruses in the winter months in a northern-hemispherical pattern of circulation, with minimal circulation in 2011 and 2016 but contributing significantly to the circulation during other seasons.

The relative contribution of the two lineages of influenza B to the yearly circulation can be seen in Fig. 2. Overall 35.4% (n = 106/299) were of Victoria lineage whereas 53.8% (n = 161/299) were of Yamagata lineage. While the dominant circulating strain matched with the vaccine strain, a significant part of the circulation was of a different strain (Figs. 2, 3). Subtyping was not possible in 32 samples, 27 in the first year due to paucity of reagents and subsequently 5 samples due to insufficient samples.

Of the 14 representative samples, 11 (2012: 4/5, 2013: 2/4, 2014: 5/5) were successfully propagated by inoculating the clinical specimens on MDCK cells. The hemagglutination activity of all isolates tested was inhibited by ferret antisera raised against reference viruses of 2010–2015. The HI titers of these antisera against the isolates tested were equivalent or within fourfold reduction as compared to homologous titer of each reference virus (data not shown). These results indicate that all the study viruses were antigenically similar to the respective circulating type B lineages. Phylogenetic analysis showed that in 2013 and 2014 Yamagata strains were in circulation. The 2013 strains were similar to respective vaccine component i.e. B/Wisconsin/01/2010 which was the vaccine component for 2012–2013. The 2014 strains were similar to B/Massachusetts/02/2012 which was vaccine component for 2013–2014 and 2014–2015. In 2012 the Victoria lineage viruses were in circulation and were similar to B/Brisbane/60/2008 which was vaccine component from 2009 to 2012. On the basis of HAI assay, circulating viruses remained antigenically similar to the respective vaccine component. Of the 299 cases, 30 (10%) required hospitalization and received oseltamivir. Of these, 4 expired whereas others had an uneventful recovery.

Our data demonstrate that influenza B co-circulated with other influenza viruses, with varying contribution, during the influenza seasons of 2010 till 2016 in Kashmir. We have previously also demonstrated influenza B to be responsible for seasonal influenza associated ARI in

Fig. 1 Graphical representation of the percentage various influenza subtypes in different years (2011–2016)

Table 1 Showing the symptoms in influenza B positive and influenza negative patients

Symptoms	Influenza B positive N = 299 N(%)	Negatives N = 5521 N(%)	<i>p</i> values
Fever	288 (96.32)	4998 (90.5)	0.0007
Chills	234 (78.26)	3604 (65.2)	< 0.0001
Nasal discharge	212 (70.9)	3690 (66.8)	0.14
Ear discharge	8 (2.67)	75 (1.35)	0.06
Cough	278 (92.9)	4936 (89.4)	0.05
Sore throat	194 (64.8)	2867 (51.9)	< 0.0001
Breathlessness	189 (63.2)	3844 (69.6)	0.0195
Expectoration	139 (46.4)	2461 (44.57)	0.52
Headache	187 (62.5)	2850 (51.6)	0.0002
Body-ache	200 (66.8)	2925 (52.9)	< 0.0001
Fatigue/malaise	206 (68.8)	3283 (59.4)	0.0012
ARI in family	69 (23.0)	934 (16.9)	0.006
Concomitant	15 (5.0)	457 (8.2)	0.05
Vomiting/nausea	28 (9.36)	1108 (20.0)	< 0.0001
Diarrhea	21 (7.0)	597 (10.81)	0.04
Seizure	0 (0.0)	34 (0.61)	0.18
ILI in family	16 (5.35)	195 (3.53)	0.10
Recent visit outside India	14 (4.68)	322 (5.83)	0.38
Influenza immunization	12 (4.0)	281 (5.09)	0.44

Kashmir as well as being responsible for an interseasonal outbreak in a nomadic area located at a high altitude [8]. The overall prevalence of 4.4% is in general conformity with a recent study of 1.5 million samples seen over 2007–2015 where more than 0.3 million samples testing positive for influenza viruses. Of these about 29,319 samples were from India, 11.8% ($n = 3471$) tested positive for influenza viruses; 1313 (4.5%) positive for influenza B, constituting about 37% of the total positives. Some of the seasons in the report had an exclusive circulation of influenza B.

The influenza B activity in our study had a pattern that was similar to the circulation of Influenza A, in conformity with the latitude related patterns of circulation reported recently by Saha et al. [18]. Located north of the 30°N latitude, the circulation of influenza B in Kashmir conforms to the pattern of influenza circulation previously described by us [4, 18] with dominant circulation during the winter months and a minimal circulation during other months of the year. Despite its location in the northern hemisphere, the seasonality of influenza in India has sub-regional variations and we have postulated reported latitude related differences in seasonality, with regions north of the 30 degree North latitude having a typical Northern hemispherical seasonality whereas regions located south of this latitude exhibiting a Southern hemispherical type of seasonality with peaking in the summer months, also coinciding with the monsoon rains in the regions [10]. Saha et al. [18] in their recent study of tropical Asian countries,

reported that in areas lying north of the 30°N latitude (northern China, Japan) high level of influenza A activity was seen during the winter months of November–March whereas areas between 10° and 30°N latitude (India, Thailand, southern China, Cambodia, Lao PDR, Philippines) increased activity of influenza was seen during monsoon months of June–December although some areas (southern China, Thailand, India,) showed a low level (3–8%) of circulation even during non-epidemic months. For areas lying close to the equator (0–10°N latitude like Malaysia and Singapore), no specific seasonality for influenza A virus circulation was demonstrable. For influenza B two patterns of circulation were demonstrable. In areas lying north of the 30°N latitude (Japan and northern China), the seasonality of influenza B coincided with that of influenza A with northern hemispherical pattern of circulation whereas in areas south of the 30°N (except Cambodia), a year-round circulation with 3–8% of annual influenza B positives during most months. Temperate geography and climatic factors might have a role to play because winters are cold and severe in Kashmir similar to those seen in northern hemispherical areas depicting a similar pattern of influenza seasonality as against the more warm and subtropical climate of the rest of the country where a different seasonality is demonstrable [4].

Our data also show that there was a co-circulation of the 2 lineages of influenza B with dominance of either of the lineages and the other constituting up to 37% of the circulation of influenza. The two lineages of influenza B

Fig. 2 a Graphical representation of the proportion of different influenza subtypes among the positive cases. **b** The relative percentage distribution of the Yamagata and Victoria lineages in different years (2010–2016). The vaccine strains for the trivalent vaccine contained 2011–2012—B/Brisbane/60/2008-like virus (B/Victoria), B/Wisconsin/1/2010-like virus for 2012–2013, B/Massachusetts/2/2012-like virus for 2013–2014 and 2014–2015 seasons (Yamagata lineage) and B/Phuket/3073/2013-like virus (Yamagata lineage)

viruses are currently believed to have diverged approximately 40 years ago and have since co-circulated on a global scale, despite frequent reassortment among them [5]. A recent report on the surveillance from the tropical Asian countries demonstrated that influenza B B/Yamagata-lineage viruses predominated during 2007–2008, and B/Victoria-lineage during 2009–2010 and co-circulation of both lineages during 2012. B/Yamagata-lineage again became the predominant strain in 2013 [18]. Co-circulation of the 2 lineages of influenza B has been reported from other geographical areas as well [2, 3, 17, 19]. While Indian data has not specifically focussed on influenza B, recently co-circulation of both lineages has been reported from Eastern India with 244 influenza B of the 3035 influenza positive revealing co-circulation of B/Yamagata and B/Victoria lineages [17]. An in-depth study of previous surveillance reports also obtusely suggests a co-circulation of the 2 lineages of influenza B in India although these studies were not primarily designed to address the issue [4, 14].

As can be seen from Fig. 3, the vaccine strain contained in the available trivalent inactivated influenza vaccine matched with the dominant circulating B-lineage. However, the co-circulation of non-vaccine lineage of the B virus in a significant proportion would make a case for additional protection by a quadrivalent vaccine in contrast to the trivalent vaccine currently approved for use in India [1, 3]. According to the The Global Influenza B Study based on data from 26 countries in both hemispheres and the inter-tropical region, the average rate of influenza B lineage mismatch with the vaccine strains was about 25% [2]. Quadrivalent vaccine (QIV) has been found to be equi-immunogenic and safe when compared to trivalent inactivated vaccine (TIV) [12]. In a recent meta-analysis of 5 randomized studies, QIV was found to be as immunogenic as TIV against the shared three strains included in the TIV with a superior efficacy against the alternate B-lineage with a safety profile similar to that of the TIV [16]. It may be pertinent to emphasize that because influenza B is less

Fig. 3 Phylogenetic tree showing the relationship of Srinagar Influenza B strains with other influenza B strains, including the vaccine strain

prone to antigenic shift than influenza A, it is generally believed to be less important clinically than influenza A infection. However, influenza B epidemics occur in about half of influenza seasons globally and are thus likely to have a substantial burden on influenza related morbidity and mortality across the world. Further there are distinct seasons when influenza B continues to be the major circulating strain and as such vaccinating against both the lineages seems sagacious.

In conclusion our data demonstrate that influenza B in temperate north India (north of the 30°N latitude) co-circulates with influenza A in a northern hemispherical pattern of seasonality. There is a co-circulation of the B/Victoria and B/Yamagata lineages with variable frequency and an obvious variable vaccine match with the trivalent inactivated influenza vaccine. Our data lend weight to the consideration of the use of QIV as against the currently approved TIV to obviate the mismatch between the circulating and the vaccine influenza B strain [1]. It is also important to perform continued surveillance studies as well as studies of the development of antiviral resistance so as to tailor adequate antiviral therapy once there is documentation of the viral infection.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest regarding the manuscript.

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