Influenza surveillance in Pune, India: Reappearance of B/Victoria/2/87-like influenza virus strain in 2002

L. R. Yeolekar*, P. B. Kulkarni, S. D. Pawar and B. L. Rao

Department of Virology, National Institute of Virology (ICMR), 20-A, Dr Ambedkar Road, Pune 411 001, India

Increase in the number of acute respiratory infection cases was observed in the month of February 2002 at Pune, India. A total of 11 clinical samples were positive for influenza viral antigen and virus isolates were obtained from eight samples. Antigenic and genetic analyses of these isolates identified them to be similar to B/Beijing/243/97 belonging to the B/Victoria/2/87 lineage. B/Victoria/2/87-like strains were circulating in Pune during 1987–89, whereas strains belonging to B/Yamagata/16/88 lineage were circulating between 1990 and 2001. Isolation of B/Victoria/2/87-like strains in 2002 indicates reappearance of this strain in the community, highlighting the need for regular influenza strain surveillance for effective control measures.

WORLDWIDE epidemics of influenza A and B occur regularly and affect large segments of the world's population. Incidences of recurring epidemics of influenza are primarily attributed to the high frequency of mutational changes in the major surface glycoproteins, viz. hemagglutinin and neuraminidase. These antigenic changes lead to reduced reactivity of human antibodies to the newly circulating virus variants and renewed susceptibility to the new variant strains causing epidemics[1].

Influenza A viruses cause yearly epidemics due to antigenic drift, punctuated by infrequent pandemics following antigenic shift. Generally, one dominant strain of both subtypes A(H3N2) and A(H1N1) predominates globally. However, multiple lineages of influenza B are known to circulate worldwide over a period of time. Two antigenically and genetically distinct lineages of influenza B viruses represented by B/Victoria/2/87 (Victoria) and B/Yamagata/16/88 (Yamagata) have co-circulated globally[2–4] since 1988. Since 1991, majority of the influenza type-B viruses isolated the world over were of the Yamagata lineage with intermittent isolation of influenza type-B Victoria lineage reported only from East Asia[5–7]. Reappearance of Victoria lineage viruses outside East Asia was reported[8] during the influenza season of 2001 and 2002.

In view of the public health importance of influenza, World Health Organization (WHO) initiated a global network of surveillance centres in 1948. Influenza surveillance is carried out throughout the year at these centres, to detect new antigenic variants emerging in the community. Monitoring the antigenic drift of viruses in global populations is essential for optimal selection of component strains for the annually updated trivalent influenza vaccine.

Influenza surveillance was initiated at the National Institute of Virology (NIV), Pune in 1976, with the objective of early detection of newly emerging strains in the community and generation of regional epidemiological and virological data. Surveillance data are essential for implementation of effective intervention programmes. During the course of continuous surveillance on influenza in Pune between 1976 and 2001, several outbreaks of influenza were investigated. In Pune, which has tropical monsoon climate, influenza outbreaks predominantly occur during the rainy season. However, some increase in the influenza activity has also been shown in the months of February–April[9]. Strains of influenza B belonging to the Victoria lineage have been isolated[9] in Pune between 1987 and 1989. However, all the type-B strains isolated between 1990 and 2001 belonged to the Yamagata lineage[10,11].

During February and March 2002, increase in the number of cases with acute respiratory infection was observed. A total of 89 throat/nasal swab and 32 nasopharyngeal aspirates (NPA) were collected in transport medium from patients with acute respiratory infection attending the pediatric outpatient departments of Sassoon General Hospital, Yerwada Corporation Dispensary and KEM Hospital, Pune. Patients with symptoms of fever accompanied by sore throat, running nose and/or cough were included in the study.

For virus isolation and identification, MDCK cells were grown in shell vials and inoculated with 300 µl of clinical specimen in the presence of viral growth medium (minimal essential medium with 2 µg/ml crystalline trypsin) centrifuged at 2000 rpm for 45 min and incubated at 35°C. Cells were observed for 7 days and harvested when cytopathic effect was evident. Supernatants from all shell vials were subjected to hemagglutination (HA) test using standard method. Strain identification of HA-positive isolates was performed by hemagglutination inhibition (HI) test according to the method prescribed by Kendal *et al.*[12]. Strains are grouped under one lineage, when they react with immune sera of a particular lineage with fourfold higher titres compared to other lineage.

All clinical specimens were screened for the presence of influenza antigen in indigenously developed antigen capture ELISA. Briefly, 1 : 100 dilution of anti influenza A and B monoclonal antibodies supplied by WHO were coated on microtitre wells in carbonate buffer and incubated at 4°C overnight. Wells were post-coated with 1% bovine serum albumin in phosphate buffered saline, 100 µl of clinical sample was added to each of the anti influenza A and B-coated wells and incubated at 37°C for 3 h. Anti influenza A and B rabbit polyclonal antibodies were added to the respective wells and incubated at 37°C for 1 h. To each well, 100 µl of anti rabbit HRP conjugate

*For correspondence. (e-mail: icmrniv@icmrniv.ren.nic.in)

(Sigma, USA) was added and incubated at 37°C for 1 h. O-phenylene diamine, 400 µg/ml and urea peroxide, 200 µg/ml were used as substrate and the reaction was stopped with 4N H_2SO_4. Wells were read at 492 nm in a Titertek ELISA reader and wells with optical density ratios of clinical sample to negative control greater than two were scored as positive. Exudate cells from NPA were screened for the presence of viral antigen in an immunofluoresence (IF) test using respiratory panel 1 viral screening and identification kit (Light Diagnostics, Chemicon, USA), essentially according to the manufacturer's instruction.

Screening of clinical samples by virus isolation and antigen detection identified influenza B virus in 11 (9%) of the 121 samples collected, 10 during February and one during the first week of April 2002 (Figure 1). Virus isolates were obtained from eight samples; two samples were positive only in ELISA test and one was positive only in IF test. Among the eight isolation-positive samples, two were positive in ELISA and two were positive in IF test. In all, four samples were positive in ELISA and three were positive in IF test. The age of children showing presence of influenza antigen ranged from 10 months to 8 years, with an average of 3.75 years.

Strain identification of all eight isolates by HI test revealed the strains to be similar to B/Beijing/243/97 belonging to the Victoria lineage of type-B influenza virus. Two representative isolates were sent to WHO Collaborating Center on Influenza Reference and Research, Atlanta, USA for genetic analysis. They were identified by the Center as being similar to B/Beijing/243/97 genetic group by restriction fragment length polymorphism. This indicates that strains isolated during the year 2002 were of the Victoria lineage, similar to the strains that circulated during 1987–89, and antigenically distinct from the strains of the Yamagata lineage isolated between 1990 and 2001. This heralds the reappearance of the strains from the Victoria lineage in Pune causing illness in pediatric patients, similar to that observed the world over. Isolation of B/Victoria/2/87-like viruses from other areas of India has been reported[8] in 2001. Sequence analysis of representative strains circulating globally in 2000–02 belonging to the B/Victoria lineage has been reported to fall into two clades[8], one similar to the B/Beijing/243/97 strain and other similar to the B/Taiwan/217/97 strain. However, though viruses from these two clades are phylogenetically distinct, they are antigenically similar[8].

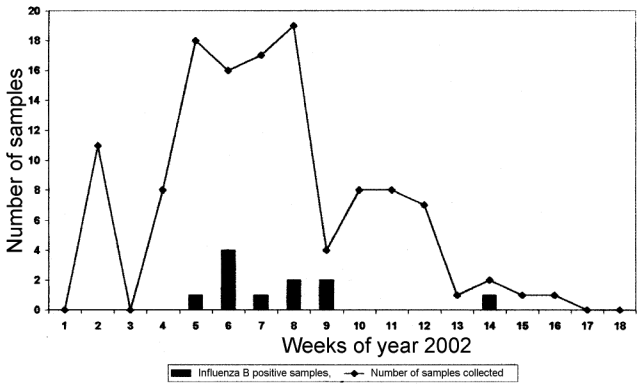

Figure 1. Week-wise collection of samples from cases with acute respiratory tract infection and detection of influenza type-B virus during January–May 2002.

To study the antigenic cross-reactivity between the two lineages isolated in our laboratory, 11 representative isolates of influenza type-B virus previously isolated at our institute since 1989 (Table 1) and identified as belonging to either Victoria or Yamagata lineage and two isolates from year 2002 (reported in the present communication) were tested using the HI test. The reference anti-sera used in the test were B/Victoria/2/87(B/Vict/87) and B/Beijing/243/97(B/Beij/97) of the Victoria lineage, and B/Yamagata/16/88 (B/Yama/88) and B/Beijing/184/93 (B/Beij/93) of the Yamagata lineage, obtained from WHO.

As indicated in Table 1, in the HI test influenza B viruses isolated in the years 1989 and 2002 showed absence or low reactivity with anti-sera raised against B/Yama/88 and B/Beij/93, the Yamagata lineage viruses. All the type-B strains isolated from 1990 to 2001 reacted with high titres to anti-sera raised against B/Yama/88 and B/Beij/93 and low titres to B/Vict/87 and B/Beij/97, indicating lack of cross-reactivity. Hence, even though an age-stratified sero-survey conducted in Pune between 1997 and 1999 showed the presence of antibodies (which increase with age) in the range of 1–26% and 5–17% to B/Vict/87 lineage and B/Yama/88 lineage respectively[13], immunity to Yamagata lineage would not render protection from Victoria lineage and vice versa. Why the Victoria lineage viruses were confined to East Asia for the past ten years and why they have now spread globally is not known. However, one can surmise that reduced reactivity of human antibodies to a variant strain and increase in the number of children immunologically naive to influenza may lead to renewed susceptibility. Re-introduction of previous strains can occur in such susceptible population and cause epidemics.

Annual vaccination is the primary means for reducing the impact of influenza. The currently used influenza virus vaccines are formulated to include only a single influenza B virus in their preparation. During periods when two antigenically distinct lineages are circulating, WHO recommendation for influenza vaccine preparation proposes the use of any one lineage depending on local conditions[14,15]. Under such circumstances, identification of influenza strains circulating locally is the key factor for proper vaccine formulation. Our surveillance has shown reintroduction of influenza type-B belonging to the Victoria lineage after a gap of nearly 12 years, highlighting the need for regular influenza strain surveillance locally in all the countries considering the introduction of influ-

Table 1. Hemagglutination inhibition reactions of influenza B strains isolated between 1989 and 2002 with reference anti-sera of Victoria and Yamagata lineage

Strain #	Previously identified lineage	Year of isolation	HI titres* with reference sera raised against			
			B/Vict/87 (Victoria)[#]	B/Beij/97 (Victoria)	B/Yama/88 (Yamagata)	B/Beij/93 (Yamagata)
893015	Victoria	1989	>320	80	10	10
8914306	Victoria	1989	80	>320	20	<10
907119	Yamagata	1990	40	20	>320	>320
9012215	Yamagata	1990	80	<10	>320	>320
934855	Yamagata	1993	40	80	>320	>320
956087	Yamagata	1995	20	<10	160	>320
965138	Yamagata	1996	20	<10	>320	>320
985504	Yamagata	1998	10	<10	80	160
98193	Yamagata	1998	40	<10	160	160
003347	Yamagata	2000	20	<10	160	>320
0198	Yamagata	2001	20	<10	80	160
021118	Victoria	2002	40	40	10	<10
021541	Victoria	2002	40	80	<10	<10

*Titres are presented as reciprocal of the highest dilution showing complete hemagglutination inhibition.
[#]Influenza type-B lineage.

enza vaccine. Only routine surveillance would ensure proper and effective implementation of control measures.

1. Glezen, W. P. and Couch, R. B., In *Viral Infections of Humans. Epidemiology and Control* (eds Evans, A. S. and Kaslow, R. A.), Plenum Medical Book Company, New York, 1997, pp. 473–506.
2. Rota, P., Wallis, T. R., Harmon, M. W., Rota, J. S., Kendal, A. P. and Nerome, K., Co-circulation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology*, 1990, **175**, 59–68.
3. Kanegae, Y. *et al.*, Evolutionary pattern of the haemagglutinin gene of influenza B viruses isolated in Japan; co-circulating lineages in the same epidemic season. *J. Virol.*, 1990, **64**, 2860–2865.
4. Rota, P. A., Hemphill, M. L., Whistler, T., Regnery, H. L. and Kendal, A. P., Antigenic and genetic characterization of the haemagglutination of recent co-circulating strains of influenza B virus. *J. Gen. Virol.*, 1992, **73**, 2737–2742.
5. WHO recommended composition of influenza virus vaccines for use in the 1992–1993 season. *Wkly. Epidemiol. Rec.*, 1992, **67**, 57–60.
6. WHO recommended composition of influenza virus vaccines for use in the 1997–1998 season. *Wkly. Epidemiol. Rec.*, 1997, **72**, 57–61.
7. WHO recommended composition of influenza virus vaccines for use in the 1998–1999 season. *Wkly. Epidemiol. Rec.*, 1998, **73**, 56–61.
8. Shaw, M. W. *et al.*, Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000–2001 and 2001–2002 seasons. *Virology*, 2002, **303**, 1–8.
9. Rao, B. L. and Banerjee, K., Influenza surveillance in Pune, India 1978–90. *Bull. WHO*, 1993, **71**, 177–181.
10. Rao, B. L. and Kadam, S. S., Isolation of recent variant influenza type A (H3N2) and B strains in Pune, India during 1998. *Indian J. Med. Res.*, 2000, **111**, 3–5.
11. Rao, B. L., Kadam, S. S. and Pawar, M. S., Isolation of recent variant influenza types A (H3N2), A(H1N1) and B strains in Pune, India. *Indian J. Med. Res.*, 2001, **114**, 157–159.
12. Kendal, A. P., Pereira, M. S. and Skehel, J. J., In *Concepts and Procedures for Laboratory-based Influenza Surveillance*, Centre for Disease Control and Prevention, Atlanta, GA, USA, 1982, pp. B17–B24.
13. Yeolekar, L. R., Kulkarni, P. B., Chadha, M. S. and Rao, B. L., Seroepidemiology of influenza in Pune, India. *Indian J. Med. Res.*, 2001, **114**, 121–126.
14. WHO recommended composition of influenza virus vaccines for use in the 1999–2000 season. *Wkly. Epidemiol. Rec.*, 1999, **74**, 57–61.
15. WHO recommended composition of influenza virus vaccines for use in the 2000 season. *Wkly. Epidemiol. Rec.*, 1999, **74**, 321–325.

ACKNOWLEDGEMENTS. We are grateful to the Director and staff of the WHO Collaborating Centre on Influenza Reference and Research, Atlanta, USA; the Medical Officers of Sassoon General Hospital (Paediatrics Department); Yerwada Corporation Dispensary and KEM Hospital, Pune and Director and staff of the Virology Section, NIV for their help and cooperation during this investigation.

Received 31 July 2003; revised accepted 11 November 2003