

# Human Influenza

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## 7 Human Influenza

- Epidemiological and virological monitoring of human influenza viruses in India
- Sensitivity testing of Real time PCR for seasonal Influenza viruses
- WHO External Quality Assessment program for the detection of Type A Influenza
- Outbreak Investigations
- Circulating Genotypes of Human Respiratory Syncytial Virus (HRSV) in Pune
- Studies on Human metapneumo virus (hMPV)



## Epidemiological and virological monitoring of human influenza viruses in India

MS Chadha

This is an ongoing multi-centric surveillance project, funded by ICMR- CDC. Influenza surveillance is carried out by 9 centers at different geographical sites in India. NIV is one of the regional centers and also the Referral center. Each regional center is responsible for identification of patients with acute respiratory infection and collection of clinical samples from these cases. Further, virus is processed for isolation and identification of circulating strains of influenza is carried out. NIV is carrying out influenza surveillance in and around Pune. The referral center receives isolates from all 9 regional centers for antigenic confirmation by way of re-characterization of isolates. Further, genetic analysis is also carried out.

### Objectives

- To establish epidemiological and virological influenza surveillance network in different geographical areas of India.
- Detection of new strains of the virus & contribution of influenza strains and information generated to the global influenza surveillance network.
- Human resource development through training and strengthening of infrastructure.
- Timely dissemination of information generated and improvement of awareness

### Activity of the regional center

During the year of reporting, surveillance activity for influenza was continued. 547 patients with acute upper/lower respiratory infections conforming to the case definition were identified. Clinical specimens (throat swabs (246), nasal swabs (247) and 4 nasal pharyngeal aspirates) were collected from them. The above included 13 samples received from pediatricians and general practitioners from Mumbai. All the samples were processed for virus isolation in MDCK cell line. Thirty nine specimens yielded virus isolates, twenty two were identified as Influenza A (H1), 11 as A(H3) and six were identified as type B. Remaining 20 samples are yet to be processed.

### Activity of the referral center

#### Antigenic characterization of isolates received from regional centers

##### Kolkata

##### Lot 1:13 Isolates- Received in May 07

Thirteen specimens (tissue culture fluid) were inoculated into MDCK cell line and processed for antigenic characterization. Seven isolates were positive for virus isolation out of which two were A (H1), 2 were A (H3), and 3 were Type B. Out of which two were Yamagata lineage, and 1 was Victoria lineage. Five specimens were negative for isolation; Remaining one isolate could not be typed due to low HA titre.

##### Lot 2: Received in October 07

Thirty isolates were received from Kolkata. All were processed for virus isolation. Twenty four isolates were positive. Out of which 18 were typed as A (H1), Five isolates were typed as A(H3) and one isolate was typed as Type B. 2 samples showed discrepancy. Remaining Four isolates were negative

## **Lot 3: Received in March 08**

Eleven isolates received from Kolkata were processed for virus isolation and Eight yielded isolates, antigenitically similar to B/Shanghai/361/02 (Yamagata lineage). Three samples were negative for isolation and by PCR.

## **Delhi:**

### **Received in August 07**

Twenty isolates from AIIMS were received. These were re-grown in MDCK. 16 isolates were positive, Fifteen were re-confirmed as A (H1), one as Type B. Remaining Four isolates did not grow up to sufficient HA titer, but could be confirmed by RT-PCR.

## **Chennai:**

### **Received in August 07**

Total eighteen isolates were received from Kings Institute Chennai. One isolate was re confirmed as A (H1). seventeen isolates were negative for isolation. Out of these seventeen, six samples were processed for type and subtype specific PCR. All six isolates were reconfirmed as three Type B and 3 A (H1). Remaining eleven isolates were exhausted

## **Vellore**

### **Lot 1: Received in October 07**

Nine isolates were received in October 07; from Vellore regional center studying hospitalized cases with respiratory disease. Attempts to re-grow in MDCK cell line were made; Seven isolates were positive. Three isolates reconfirmed as type B, Four isolates were typed as A (H3). Remaining two isolates could not be typed due to low HA titre. These two isolates were confirmed by PCR as A (H3)

### **Lot 2: Received in March 07**

Nineteen isolates were received from CMC Vellore. All samples were processed in MDCK cell line, two were found to be Influenza A(H3), one A(H1) and sixteen isolates were Type B. positive of which ten were similar to B/Malaysia/2506/04 (Victoria lineage) and six isolates were similar to B/Shanghai /361/02(Yamagata lineage).

## **Dibrugarh**

### **Lot 1: Received in October 07**

Fifteen isolates and ten corresponding clinical samples were received. All isolates and clinical samples were passed in MDCK and all were negative. Fourteen isolates (one specimen exhausted) were also tested in RT-PCR using diagnostic primers for A and B. All fourteen were negative by PCR. On request, out of fifteen, ten corresponding clinical samples were received. There was no virus yield from these samples.

### **Lot 2: Received in November 07**

Six isolates were received. All isolates were passed in MDCK and all were negative. All isolates were subjected to diagnostic Type A, and B conventional RT-PCR, followed by sub type specific conventional two-step RT-PCR. Out of 6 Dibrugarh isolates, four were positive for Type A influenza and remaining two samples were negative in nested PCR. Sub type specific RT-PCR for four isolates from Dibrugarh was negative.

### **Lot 3: Received in February 08**

Ten isolates and corresponding clinical samples were passed in MDCK and all were found to be negative for isolation. PCR could not be performed due to insufficient sample quantity.

**VP Chest Institute (Delhi)****Lot 1: Received on Aug 07**

Ten isolates with corresponding clinical samples were received from VP Chest Delhi. All isolates/ clinical samples were passed in MDCK cell line. Out of them two pairs were A (H3) and remaining eight pairs of samples were negative for virus isolation.

All the above ten pairs of isolates and clinical samples were processed for diagnostic Type A, and B conventional RT-PCR, followed by sub type specific conventional two-step RT-PCR. Out of them, two were A (H3) by PCR (also found positive for isolation), an additional sample was Positive for Flu A but could not be subtyped in PCR. seven samples were negative for both isolation and PCR.

**Lot 2: Received on Feb 08**

Twelve isolates with corresponding clinical samples were received from VP Chest Delhi. All isolates and clinical samples were passed in MDCK cell line. All were negative for virus isolation. All isolates were subjected to Type A, and B conventional RT-PCR, followed by sub type specific conventional two-step RT-PCR. seven isolates were confirmed as Type A out of them, two isolates were subtyped as H3.

**Antigenic characterization of clinical samples received from regional centers**

1. Thirteen clinical samples were received from NICED Kolkata. All samples were processed for virus isolation in MDCK. Four samples were positive for isolation. Out of which two isolates were confirmed as Type B (Victoria lineage), one was confirmed as A (H1) and one as A (H3). Remaining 9 samples were negative. Earlier, these samples had been found positive for Influenza by RT- PCR at NICED.
2. Since the Nagpur laboratory was not fully functional, 164 clinical samples were received from Nagpur. On processing in MDCK cell line, Seven samples were positive for isolation, Five yielded Influenza A (H1), one A (H3) and one Type B.

**Genetic Analysis****i) Phylogenetic analysis of type A (H1N1) influenza viruses**

A total of 107 A (H1N1) isolates (Pune 36, Nagpur- 4, Chennai- 29 and Kolkata-26, Delhi-11, Dibrugarh-1) were analyzed by sequencing 984 nucleotide region in the HA1 gene. Analysis of the nucleotide sequences encoding the HA1 peptide region (979 bp) showed that strains circulating in 2005-2007 were clustered in two groups .One group comprised A/New Caledonia /20/99like viruses and another group comprises A/Solomon Island /3/06 or A/Brisbane/59/2007 like viruses. 23 isolates (2005) and 22 isolates (2006) were genetically closer to A/New Caledonia /20/99 (2001-2007 vaccine component). Remaining 13 isolates (2006), 6 isolates (2005), 3 isolates (2007) were genetically closer to A/Solomon Island /3/06 (2007-2008 vaccine component) and 40 (2007) isolates were genetically close to A/Brisbane/59/2007 (2008-2009 vaccine component) (**Fig.1**).

**ii) Phylogenetic analysis of type A (H3N2) influenza viruses:**

Forty Six A (H3N2) isolates (Pune- 14, Kolkata- 4, VP Chest-2, Andaman-1, Vellore 7, Nagpur 1, Delhi-10, Chennai- 3, Dibrugarh-4) were analyzed by sequencing the 984 nucleotide region in the HA1 gene. Phylogenetic analysis indicated that the circulating strains are well matched with the vaccine recommendation. All 2006 strains of H3N2 were close to A/Wisconsin (2006-2008 Vaccine component) and 2007 strains were close to A/Brisbane (2008-09 vaccine component). Two 2007 VP chest Delhi isolates were clustered with A/Panama 99. Further quality control testing was essential to rule out lab contamination but could not perform the test, as original clinical sample was not

available from Delhi center (Fig. 2)

### iii) Phylogenetic analysis of type B influenza viruses

36 Type B isolates from India were processed in this year for molecular analysis of HA gene by RT-PCR and nucleotide sequencing. These include 14 isolates from Pune, 15 from Kolkata, 3 from Vellore, 2 from Chennai and one each from Delhi and Nagpur. Sequence analysis of 855 bp region of HA1 gene was carried out.

Phylogenetic analysis clearly showed that both the lineages (Victoria and Yamagata) were circulating during the year 2007. 5 isolates from year 2005 clustered as Victoria lineage and were closely related to the vaccine strain B/Malaysia/2506/2004 (for years 2006-2008). 3 out of 4 isolates from year 2006, clustered in Victoria lineage were found to be closer to B/Malaysia/2506/2004, whereas 1 isolate was clustered in Yamagata lineage and was closer to the vaccine strain B/Shanghai/361/2002 (for years 2004-2006). 27 isolates from year 2007 were processed and it was found that 21 isolates were from the Victoria lineage and were closely related to vaccine strain B/Malaysia/2506/2004. Remaining 6 isolates clustering in Yamagata lineage were closer to vaccine strain B/Florida/4/2006 (for year 2008-2009). WHO recommended B/Florida/4/2006 (Yamagata lineage) as Type B vaccine component for the year 2008-2009. In India, during the year 2007, predominant circulating lineage was

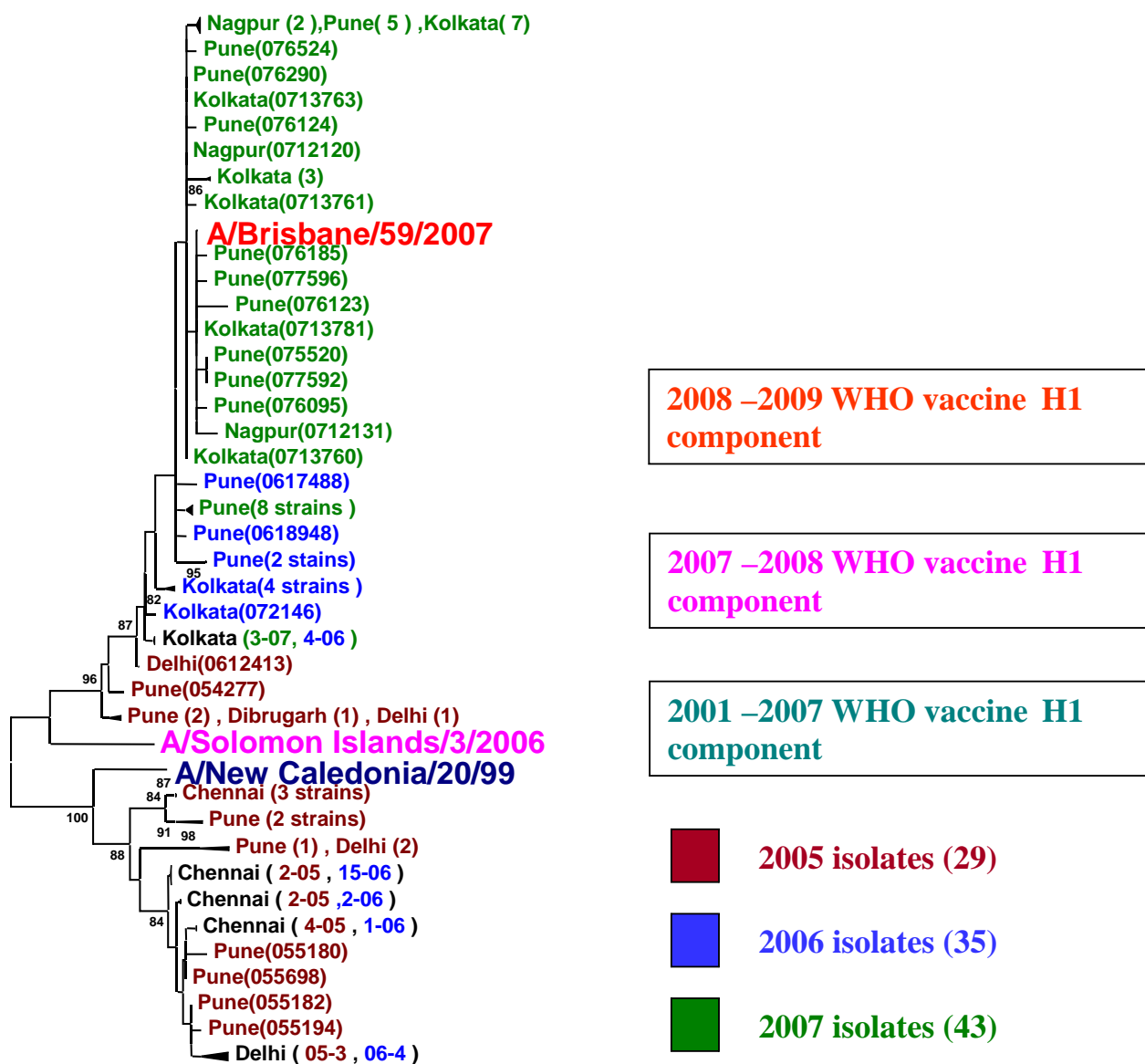


Fig 1. HA1 sequencing of 107 H1 isolates (979 bp)



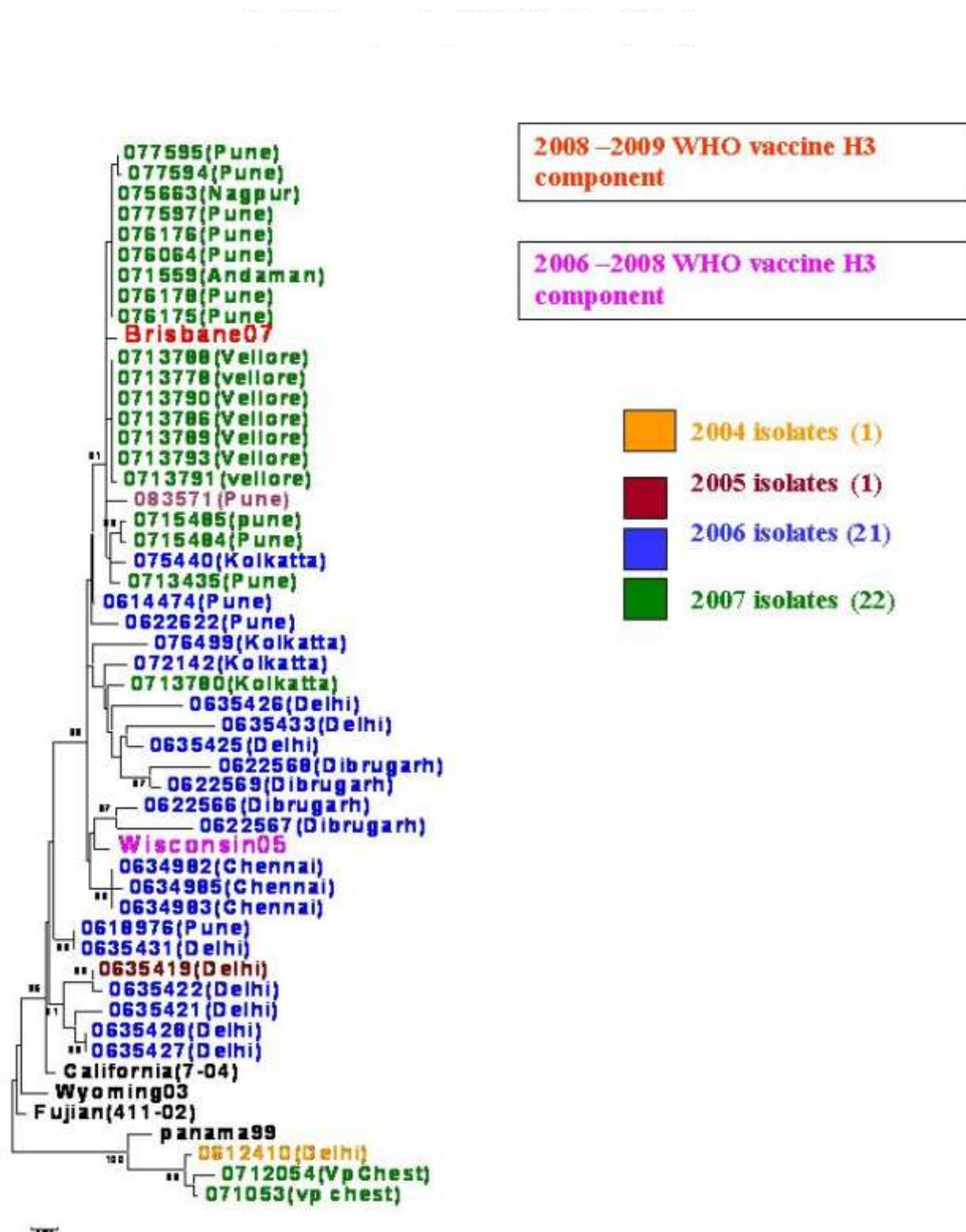


Fig. 2 : HA1 sequencing of 46 H3 isolates (984bp)

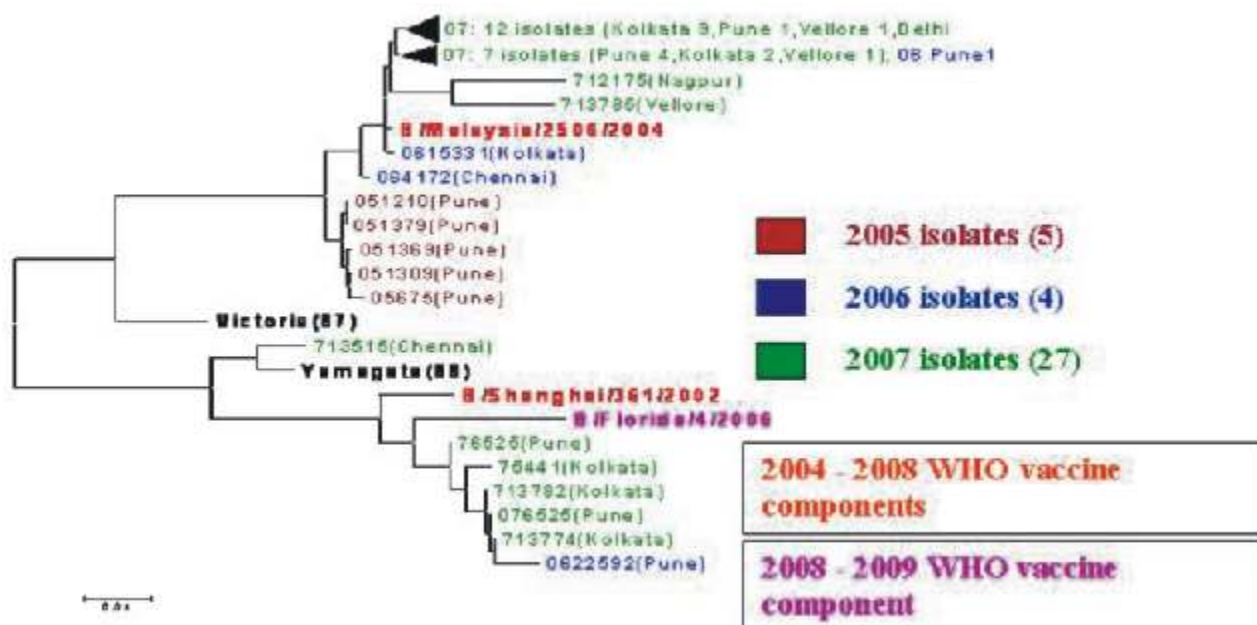


Fig. 3 : HA sequencing of 36 Type B isolates (855bp)

#### Amantadin resistance testing by M gene sequencing

A total of 59 H1 isolates and 20 H3 isolates were tested for amantadin resistance by M2 region of matrix gene sequencing. Out of twenty H3, 19 isolates showed resistance for amantadin with S31N mutation and all 59 H1 isolates and 1 H3 were sensitive for amantadin.

### Sensitivity testing of Real time PCR for seasonal Influenza viruses

VA Potdar, SR Waregaonkar and MS Chadha.

Although isolation of Influenza virus remains the gold standard for diagnosis, it is a time consuming process. Hence, the use of sensitive and quick method is desirable for the detection and sub-typing of influenza from clinical samples of patients with respiratory disease.

#### Objective

- To compare sensitivity and specificity of Real-time PCR in comparison to two-step conventional and isolation techniques for Influenza detection.

#### Work done

317 clinical samples were processed for RNA extraction followed by real time PCR for detection of Influenza type A. All these 317 samples were also processed for isolation in MDCK cell line and 140 samples for Conventional two-step PCR. 62 samples were found to be positive for influenza A by Real time PCR (CDC kit) with CT value ranging from 22 to 40. Out of these, 15 samples were found positive for both isolation as well as real time PCR, whereas only 2 samples were positive in isolation but negative in real-time PCR. 26 samples gave positive results for Conventional two-step RT-PCR. Two-step PCR for remaining 177 samples is in process.

### Future plan

Similar activity will be continued by isolation and molecular characterization of Influenza viruses from clinical samples. Diagnosis of resistance marker for NA gene will be standardized using molecular techniques.

## WHO External Quality Assessment program for the detection of Type A Influenza

VA Potdar, SR Waregaonkar and MS Chadha.

**WHO annually sends out Quality assurance Panels to participate National Influenza Centres for testing Influenza A.**

### Work done

NIV received two panels for testing

- Second Panel - received in August 2007 (14 samples)
- Third panel - received in December 2007 (10 samples) Coded samples in the panel were tested for Type A, H5, and N1 by real time PCR, one step PCR and two-step PCR. Results were 100% matched with the result sent by EQAP team.

### Future plan

To continue to participate in the said programme.

## Outbreak Investigations

MS Chadha, YK Gurav

### H5N1 Manipur Outbreak

An outbreak of avian influenza H5N1 was reported in Manipur in the month of May 2007. A total of 27 samples (Blood- 18, NPS-1, 8 TS) was collected from the contact persons with poultry and cullers. All TS and NPS were subjected to One-step RT-PCR using specific primers of HA, NA & M gene for the detection of H5, N1 and type A respectively. All 9 samples were negative for H5 and N1. 2 samples showed positive signals for type A. The two samples were further subtype for H1 and H3 using specific primers. Both the samples were positive for H1 and confirmed by sequencing

### West Bengal Avian Influenza Outbreak

An outbreak of avian flu was investigated by the NIV in February 2008. Twenty high-risk persons, i.e. cullers or people in close contact with poultry, with severe acute respiratory illness were identified. 20 human samples (throat/nasal swabs) were analyzed. These samples were processed for PCR for Influenza A/H5 (WHO protocol) and NA gene. All samples were found to be negative for Influenza A, H5 and in NA gene by PCR.

### Panchagani Outbreak

An outbreak of respiratory infection was reported from residential school in Panchagani (New Era School) in March 2008. These children were admitted / undergoing treatment at school general clinic. 53 children of age group 5 yrs to 14 yrs, having influenza like illness were examined and throat swabs from all were collected by NIV. All TS were

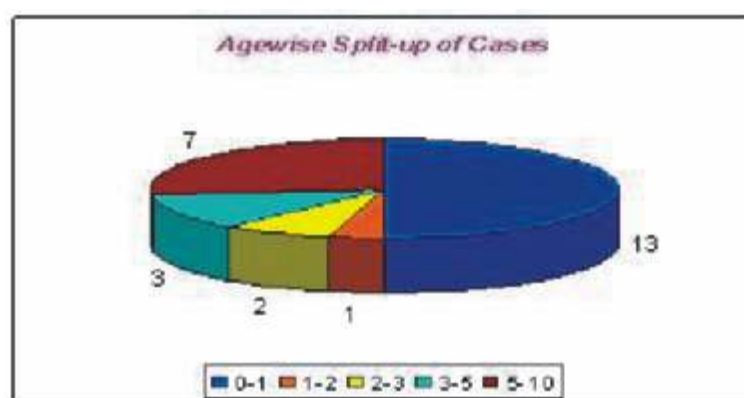
subjected to Influenza type A and Type B (diagnostic) real time PCR, followed by sub type specific real time PCR. 32 samples were positive for Flu A out of which 29 were sub-typed as H3. Four samples were positive for Type B. On antigenic analysis in MDCK cell line, 6 specimens were positive, out of which 5 were identified as Influenza A (H3) and 1 as Type B/Malaysia/2506/04 (Victoria lineage). Five H3 isolates were further analyzed for HA1 gene by sequencing. On phylogenetic analysis all five isolates clustered together with 2008 - 2009 vaccine component A/Brisbane.

## Andaman Outbreak

An outbreak of respiratory infection was reported in Andaman (G.B. Pant Hospital) in November 2007, with an increased number of pediatric hospitalizations with lower respiratory tract infections. 28 throat swabs from children of age group 3.5 months to 7 yrs, and having male female ratio of 1.5:1 were received at NIV. Case histories were available for 22 children. Presenting symptoms are shown in table 1 and age break-up in **Fig. 4**.

**Table 1 : Clinical Symptoms**

Symptoms	Number of patients
Fever not associated with rigors or chills	22/22
Cough	22/22
Breathing difficulty	14/22
Creptations	15/22
Chest in-drawing	8/22
Wheeze	4/22
Poor feeding	7/22



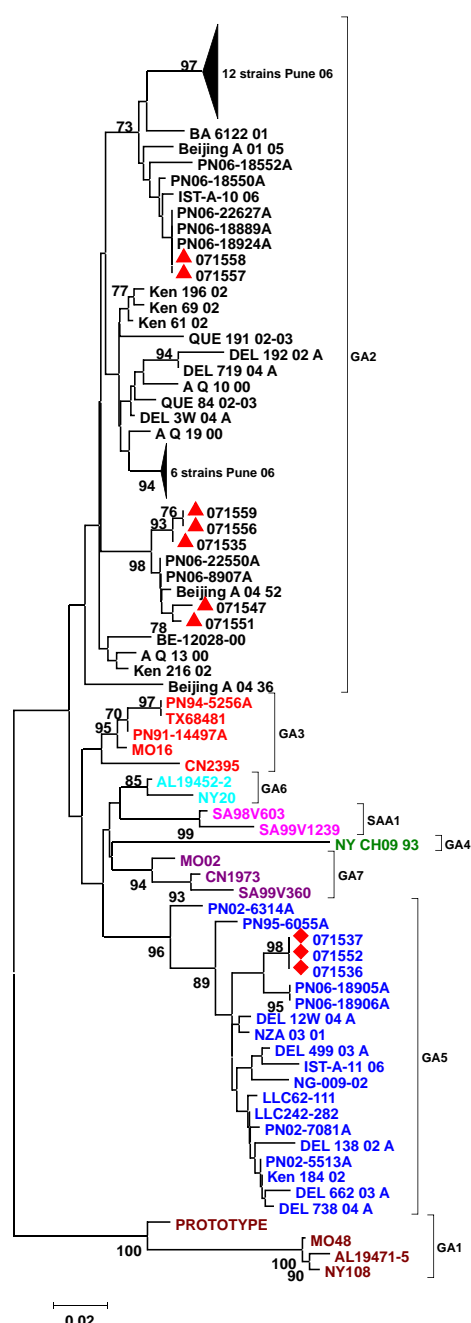
**Fig. 4 : Age-wise break up of the patients**

All the specimens were subjected to diagnostic RT-PCR for using F protein gene primers. Purified PCR products were subjected to sequencing for the confirmation of the results, where sequences from HRSV prototype strain A2, recently circulating (year 2003) subgroup A strains from Singapore and South Africa, HRSV strains (year 1996) from UK were used.

In semi nested PCR, 11/28 (30.8%) specimens were positive for HRSV. All eleven strains belonged to subgroup A of HRSV; three strains clubbed with Singapore strain LLV62-111 and eight strains with SA strain GA2SA98V173. Two patients had seasonal influenza A (H3) virus one of which showed dual infection with HRSV. Genetic analysis of A (H3) isolate showed that the strain was closely related to vaccine strain A/Brisbane 07(2008-2009)

### Phylogenetic analysis

Subgroup analysis and genotyping of HRSV strains was done using G protein gene primers where sequencing of 270-nucleotide hyper variable region at C- terminus end was sequenced. Figure 5 indicates phylogenetic analysis of HRSV strains obtained at Andaman.



**Fig. 5 : Phylogenetic analysis of HRSV strains using G protein gene**

Ten of 11 strains were genotyped. Co-circulation of genotypes GA2 and GA5 of subgroup A was observed in Andaman with GA2 being predominant.

## Circulating Genotypes of Human Respiratory Syncytial Virus (HRSV) in Pune, India

RG Damle, V Virdi and MS Chadha

Viruses are found in 20%-40% of children hospitalized with acute respiratory infections (ARIs) in India, with HRSV being one of the frequently identified viral pathogen. Previous studies at National Institute of Virology, Pune during the years 2002-2004 indicated that, 39.2% of admitted and 13.6% of pediatric patients attending out patient department of the hospital had HRSV infection. Molecular analysis of HRSV strains isolated from Pune during the year 1991 through 2004 indicated circulation of different genotypes.

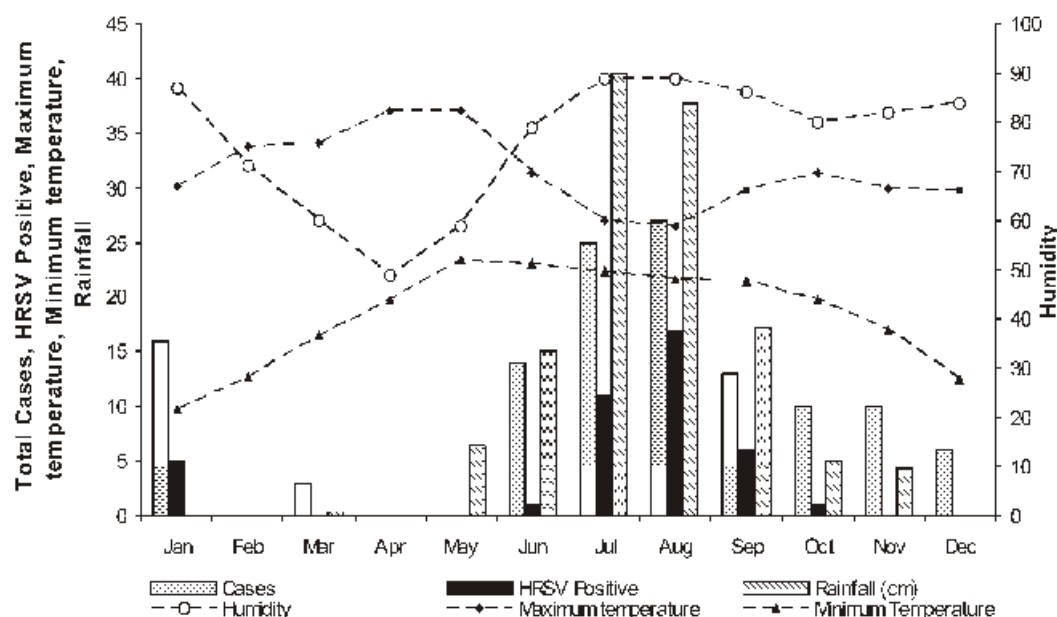
### Objective

- Molecular analysis of circulating genotypes of HRSV in pediatric patients during the year 2006

### Work Done

A retrospective study to analyze circulating genotypes of HRSV in Pune, tropical India during monsoon epidemic of 2006 was undertaken. 124 specimens from children less than five years of age were selected. These included 45 nasopharyngeal aspirates (NPA), and 79 nasal swabs (NS). Specimens were screened for HRSV positivity using F gene primers and HRSV positives were subjected to genotyping PCR using G gene primers of HRSV. The results were analyzed for HRSV positivity, phylogenetic and sequence analysis and glycosylation pattern.

Forty-one of 124 specimens (33.06%) were positive for HRSV. HRSV cases were detected from early January and the peak activity coincided with the monsoon months July through September of 2006 (**Fig. 6**).



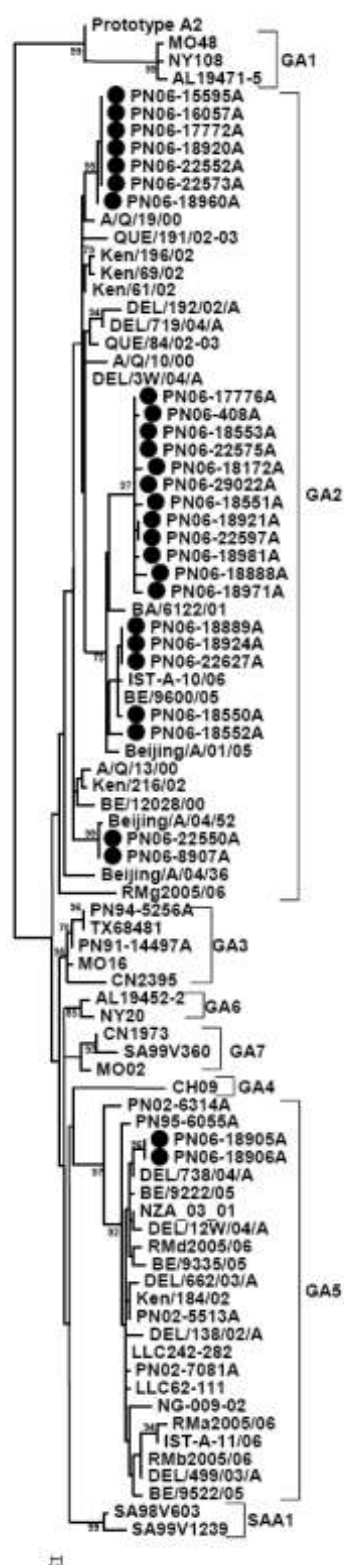
**Fig. 6 : HRSV activity in relation to climatic conditions during 2006**

Thirty-eight of 41 HRSV positive specimens could be typed. Co-circulation of both the groups A and B was observed with group A being predominant (73.68%). 26 specimens were positive for group A viruses, 10 specimens for group B and the 2 specimens had dual infection with both the groups A and B.

Phylogenetic analysis of group A viruses showed co-circulation of two genotypes GA2 (n = 26) (65%) and GA5 (n =



2), of subgroup A with genotype GA2 being predominant. Group A viruses showed identity with strains from Beijing 2005 and the strains from world over including Singapore, Turkey, South Africa, Uruguay and Kenya Pune 2006 strains were compared with group A viruses circulating in temperate regions of the world during 2005-2006 (**Fig. 7**). The Pune GA2 viruses were 11.3 % divergent at nucleotide level and 20.9 % at amino acid level, while the GA5 viruses were 14 % and 26.7 % divergent at the nucleotide and amino acid level respectively from prototype strain A2,. Within the GA2 viruses in this study, 4.1 % and 8.1 % divergence was observed at nucleotide and amino acid level respectively, while the two GA5 viruses were identical.



**Fig. 7 : Phylogenetic tree of HRSV group A viruses.**

Deduced amino acid sequences revealed GA2 viruses with two different protein length, 15 of 28 viruses had protein length of 298 amino acids while the remaining had 297 amino acid length proteins similar to GA5 viruses.

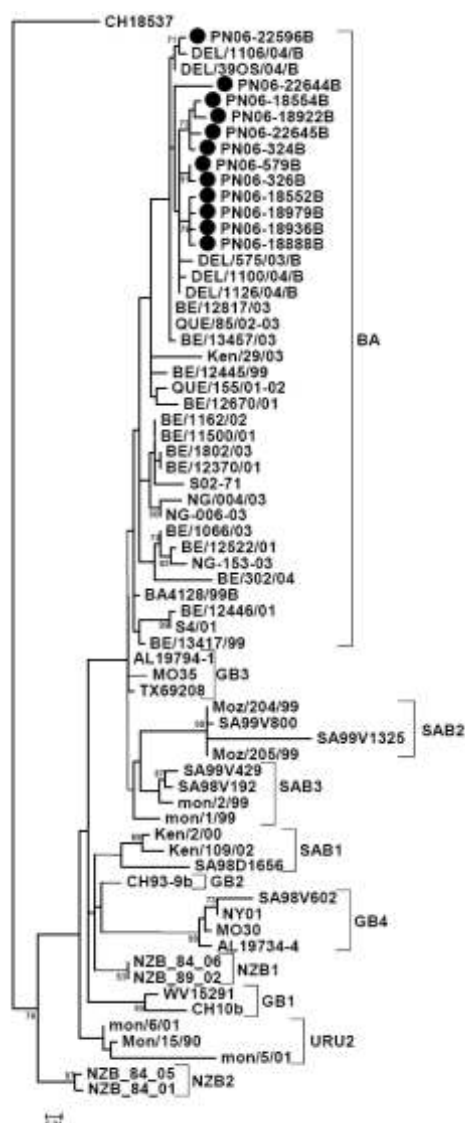
The potential N-glycosylation sequon (amino acid NXT, where X is not proline) were seen in group A strains of Pune 2006. Within the genotype GA2 two motifs (NXT) at position 237 to 239 and 251 to 253 were found similar to the prototype strain A2. An additional motif at amino acid position 294 to 296 was observed in all the genotypes except the prototype strain. The GA5 strains showed two N-glycosylation motifs one at position 237 to 239 identical to the genotype GA2, while the other found only in the GA5 strains at position 250 to 252 amino acid.

Unlike prototype strain A2 two repeats of O-glycosylation motif KPX---TTKX were present among the strains of genotype GA2. The second motif KPX---TTKX at amino acid 233 to 241 was not observed within the GA5 viruses.

From the program NetOglyc, we could predict the potential serine and threonine residues to be O-glycosylated. We could predict 20-33 such serine and threonine residues to be O-glycosylated.

### Group B viruses

All the group B viruses belonged to the genotype BA with 60-nucleotide duplication. All the 2006 Pune BA viruses clustered close together on the same branch with the 2003 strains from Canada, Belgium, and 2003-2004 strains from Delhi, India (**Fig. 8**). The BA viruses from Pune were 4 % divergent at nucleotide level and 7.3 % divergent at the amino acid level from the prototype BA strain.



**Fig. 8 : Phylogenetic tree of HRSV group B viruses**



BA viruses of two different protein lengths 312 (n = 10) and 319 (n = 2) were seen. Most of the amino acid substitutions were seen within the 20 amino acid duplication region. Within this region, the changes from S 247 P and T 270 I were seen in all the Pune 2006 strains. The change V 271 A was seen in all but one Pune strain (PN06-22596).

The change S 257 L was seen in four BA strains where as serine residue in six strains at 257 is predicted as O-linked glycosylation site using NetOglyc. The two (NXT) N-glycosylation sequon at the C terminal end were conserved among all the BA viruses of Pune 2006.

## Conclusion

Co-circulation of both the groups A (73.68%) (GA2 65% and GA5) and group B (BA) was observed with genotype GA2 being predominant with peak HRSV activity in August.

This study showed GA2 and BA viruses with two different protein lengths. Exclusive amino acid substitutions along with changes in glycosylation pattern were observed as compared to the previous studies. In four BA strains amino acid substitutions at positively selected site S 257 L and reduction in glycosylation of serine at position 267 was noticed, two of these strains showed dual infection with GA2 viruses. These changes might influence dual infection. In contrast to predominance of genotype GA5 in the recent consecutive epidemics from temperate regions, this study reports predominance of GA2 viruses. Such studies from tropical regions are necessary to understand the disparity in seasonality and predominant genotypes; essential for designing global vaccine strategies.

## Studies on Human metapneumo virus (hMPV)

VA Potdar and MS Chadha

The Human metapneumovirus (hMPV) was identified as a human respiratory pathogen in 2001, causing upper and lower respiratory tract infection in children as well as adults. HMPV has been reported from various parts of the world such as Italy, France, Spain, the UK, Germany, Denmark, Canada, the USA, also in Asia (India, Japan, China, Singapore), There are very few reports from India.

## Objectives

- The objective of this pilot study was to identify human metapneumovirus infection among patients with ARTIs by molecular diagnosis and also to determine which sub lineage was in circulation in the year 2006.

## Work done

Three Hundred and Twelve stored respiratory samples [throat swab (TS)=128, nasal swab (NS)=123 and nasopharyngeal aspirate (NPA)=47, NS+TS=14] collected from various dispensaries and KEM hospital from Pune during the year 2006 were analyzed for hMPV infection. The hMPV detection was done by diagnostic PCR using the F and N gene primers.

## Results

The presence of hMPV during 2006 was very low i.e. one case (clinical sample-NPA) among 312 ARTI patients. On F Gene analysis, positive sample clustered with the B1 sub lineage. It was reported earlier from north India that A2 lineage was in circulation during 2004-2005. The study demonstrated presence of B1 lineage first time in Pune. This preliminary study shows that the routine surveillance is required to have better understanding of seasonality

and genotype distribution of hMPV.

## Publications

- Briese T, Renwick N, Venter M, Jarman RG, Ghosh D, Kondgen S, et al. Global distribution of novel rhinovirus genotype. **Emerg Infect Dis.** 2008; 14(6): 944-947.
- Ray K, Potdar VA, Cherian SS, Pawar SD, Jadhav SM, Waregaonkar SR, et al. Characterization of the complete genome of influenza A (H5N1) virus isolated during the 2006 outbreak in poultry in India. **Virus Genes** 2008 Apr; 36(2): 345-53.

## Workshops / Conferences / Seminar / Meetings attended

### MS Chadha

- Six monthly review meeting of all centers at NIV Pune on 8th May 2007.
- Talk on "Epidemiology of Influenza in India" in the Options for the control of influenza VI at Toronto, 17-23 June, 2007
- CDC-NIV Real time PCR workshop, MCC, Pune, 8-12 October 2007.
- Talk on "Epidemiology of Influenza in India" in the International symposium on Avian Influenza: Epidemiologic, Basic and Applied Research. New Delhi- 29-31 October 2007.
- Talk on "Changing Epidemiology of Water-borne Hepatitis in India" in the II<sup>nd</sup> CMC-NIV Symposium/CME on Respiratory, Gastrointestinal, Arthropod and Blood Borne Viruses, Vellore, 6th March 2008.
- Six monthly review meeting of all regional centers and ICMR Task Force at Vellore, 6th March 2008.
- Study tour to CDC activities related to seasonal influenza surveillance and pandemic influenza preparedness, 13-14 March 2008.
- Presented Poster on "Genetic Characterization of Influenza Viruses Isolated in Western India, 2005-2007 " MS Chadha, VA Potdar, SR Waregaonkar, AC Mishra in the International Conference on Emerging Infectious Diseases, at Atlanta, 17-19 March 2008.

### PB Kulkarni

- Attended training for "Testing methods and identification of viruses" at Virus Unit, Public Health Lab Hong Kong during August September 2007.

### RG Damle

- Attended training for "Testing methods and identification of viruses" at Virus Unit, Public Health Lab Hong Kong during August September 2007.
- Attended International symposium on Avian Influenza: Epidemiological, Basic and Applied Research, New Delhi- 29-31 October 2007.

### VA Potdar

- Presented Poster on "Genetic variation in Influenza A/H3N2 virus isolates from India". VA Potdar, SR Waregaonkar, S Broor, P Gunasekharan, TN Naik, MS Chadha, AC Mishra. in the Options for the control of influenza VI at Toronto from 17-23 June, 2007
- Advanced Level Real time PCR Course organized by the Molecular Biology Technical Support and Training Center Applied Biosystems, Gurgaon, 27- 28 September 2007 .

- CDC NIV Real time workshop held at MCC Pashan from 8-12 October 2007.
- International symposium on Avian Influenza: Epidemiological, Basic and Applied Research New Delhi- 29-31 October 2007.

### **SR Waregaonkar**

- Advanced Level Real time PCR Course organized by the Molecular Biology Technical Support and Training Center Applied Biosystems, 27- 28 September 2007 Gurgaon.
- CDC NIV Real time workshop held at MCC, Pune, 8-12 October 2007.

The following Posters were presented at the International Conference On Emerging and Re- emerging Viral Diseases of the Tropic and Sub- Tropic", New Delhi, 11-14 Dec 2007.

- Influenza Surveillance in Pune, India  
Rangole MS, Kulkarni PB, Chadha MS, Mishra AC.
- Genetic analyses of influenza B viruses in India.  
Waregaonkar SR, Potdar VA, Joshi AA, Broor S, Gunasekharan P, Naik TN, Biswas D, Chadha MS, Mishra AC.
- Influenza Surveillance in Kolkata, India (2005 & 2006).  
Naik SS, Chawla M, Chadha MS, Mishra AC.

### **Technical support / consultancy provided**

- Cell lines provided to Regional Labs.
- Quality control kits to all regional labs for QA.
- Sequencing facility was provided to Dengue, Rotavirus and Measles departments and around 4000 samples were sequenced.