Executive Summary

The year 2007-08, like other years, was not spared from the challenges of addressing issues of ongoing and new viral disease outbreaks from different parts of the country. I would like to emphasize that the members of the Institute, in response, exercised their skills for planning, rapid action and strategic implementation of investigative procedures.

During the year, diverse activities were taken up in the different areas of research. However, preparedness for continuously occurring encephalitis outbreaks in the northern region and Dengue/Chikungunya outbreaks in the southern region was top priority. NIV initiated the process for establishment of its units in Uttar Pradesh and Kerala in 2007 with support from ICMR, and the health ministries of Union and State governments of Kerala and Uttar Pradesh. Encephalitis patients from Gorakhpur continued to be investigated for viral agents. Unlike the previous year, Japanese Encephalitis (JE) virus emerged as a significant cause of encephalitis although multiple types of enteroviruses were also detected. Studies to evaluate JE vaccine SA-14-14-2 were initiated in the endemic area. At Allapuzha, the Kerala Unit started actively participating in the ongoing surveillance of dengue and chikungunya viruses for better understanding of disease patterns in southern India.

NIV was instrumental in the investigation of multi district and focal outbreaks of avian influenza in West Bengal and Manipur. The aetiological agent H5N1 was successfully identified and isolated. The Avian influenza laboratory is now recognized by WHO as the H5N1 Reference Laboratory in South East Asia. As part of the Multisite Influenza monitoring activity of the Referral Centre, NIV received 157 isolates from various centers for re-characterization of virus. Of these, 64 isolates were submitted to the collaborating center at CDC, USA.

During investigations of chikungunya outbreaks in Kerala and Maharashtra, unusual manifestations involving systemic complications and deaths were reported. Based on full genome analysis of chikungunya isolates, the molecular clock, evolutionary rates and divergence times were studied in detail. In contrast to Reunion Islands, the chikungunya epidemic in India was not found to be associated with the E226V mutation in the E1 protein.

West Nile virus (WNV) activity has been detected in JE endemic areas especially in Southern India for many years as mild encephalitis, without severe sequelae. WNV strains display a spectrum of pathogenicity in mice. E protein of WNV is responsible for virus entry and is the target of neutralizing antibodies and thus would greatly contribute in pathogenicity. Sequence analysis of monoclonal antibody neutralization escape WNV mutants showed a mutation in the envelope protein of pathogenic strains rendering them nonpathogenic in mice by peripheral route.

Investigation of dengue cases in and around Pune revealed that 46% of suspected cases could be confirmed in the laboratory of which 72% were dengue fever and 28% were dengue hemorrhagic fever.

Detection of NS1 by ELISA improved early diagnosis of dengue. Studies in mice on the response of dendritic cells (DCs) to dengue virus showed that the myeloid DCs and plasmacytoid DCs modulated the immune response differentially. Studies on dengue virus morphogenesis unveiled that microtubules and intermediate filaments are important for entry and maturation of the virus. Electron microscopy and atomic force microscopy studies suggested interaction between DV and platelets.

A combined vaccine for Hepatitis E and B viruses tested in Swiss albino mice was found to be highly immunogenic. A ninth genotype-I of hepatitis B virus was identified among a primitive tribe from Arunachal Pradesh. A protocol for the detection of hepatitis A and E, rota and entero viruses was standardized for water purification systems. Generation of infectious cDNA clones for genotype 1 and 4 Hepatitis E viruses was achieved. In the field of enteric viruses, a hospital based surveillance of rotavirus disease and strains in Pune identified emergence of G12P[6] strains in children and a significant increase (6.6%- 43%) in the rotavirus positivity among adolescents and adults with rise in the infections with untypable strains. A murine model was developed for rotavirus infection and evaluation of anti-rota antibodies for passive protection. Noroviruses (NoV) were identified as the second most important cause of acute gastroenteritis after rotaviruses. Summer month seasonality supported NoV infections in western India. The Norovirus positivity rate was significantly high in patients below 2-years of age. The phylogenetic analysis of partial RNA polymerase and VP1 (capsid) genes identified 2 Genogroup I and 5 Genogroup II genetic clusters with possible occurrence of a "2007 new variant" of GII.4 and six different recombinations including 3 known and 3 novel types, thus indicating diversity in circulating NoVs. Association of enteric adenovirus serotypes 40, 41 and 31 and astrovirus serotype 8 and multiple enterovirus serotypes with acute gastroenteritis was detected for the first time in India.

Measles virus laboratory is declared as GCLP compliant laboratory for vaccine trial leading to the honor of being the first GCLP compliant ICMR laboratory. Also this laboratory is recognized by WHO SEARO as the Regional Reference Laboratory in the WHO SEAR Measles Laboratory network for virus isolation and genotyping. Measles virus genotype D7 was detected for the first time in India.

A recombinant protein-based vaccine for Chandipura virus was developed and found efficacious when administered along with DPT in a mouse model. Neuropathogenesis and immunopathogenesis of Chandipura virus was studied using murine models which proved the neurotropic nature of the virus and involvement of different cytokines in it's pathogenesis.

NIV field station at Banglore has been successfully accredited by WHO for Polio and Measles Laboratories in the WHO SEAR Laboratories network.

A diagnostic RT-PCR and Real Time PCR for a highly infectious Kyasanur Forest Disease virus were established in a high containment laboratory. This facility continued to provide support for processing of outbreak specimens of unknown etiology. Importantly, for diagnosis of JE, Dengue and Chikungunya NIV supplied 802 MAC ELISA kits to various sentinel surveillance hospitals / Apex laboratories. A validation report on JE IgM ELISA kit from CDC, Atlanta has documented 96.3% sensitivity and 95.7% specificity for testing of CSF samples.

The continuous efforts of scientists, technical, administrative and support staff for these endeavours are highly appreciable. Simultaneously, invaluable support from Director General and officials from ICMR while performing the challenging tasks, is gratefully acknowledged.

A.C.Mishra
Director

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Outbreak Response

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Mr. PA More	Technical Assistant

1 Outbreak Response

Outbreak investigations

- **Avian Influenza**
- **Influenza**
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- **Chandipura Encephalitis**

Outbreak Investigations

Avian Influenza

Avian Influenza outbreak in Imphal, Manipur

BV Tandale

Introduction

Avian Influenza in backyard poultry in Chingmeirong locality in Imphal, Manipur was investigated as a part of Central team between 27th July and 5th August 2007. The poultry deaths reported only from Chingmeirong locality, part of Imphal city, were confirmed as H5N1. Culling operations were done between 26th July and 3rd August 2007.

Objective

- Surveillance for detecting H5N1 infections in patients reporting acute respiratory infection (ARI) following natural/ occupational exposure

Work done

There were no suspect cases in two isolation wards set up in Jawaharlal Nehru Hospital and Regional Institute of Medical Sciences. Health workers detected ARI in a family with poultry deaths during survey on 27th July. Three sick members of a family with 7 Members were investigated as "persons under investigation". The fourth member of the family, aged 2 years, developed ARI, and was also sampled. On 28th July, post mortem NPS and blood were collected by State Health Department from a culler, known alcoholic and reportedly without respiratory illness. In addition, blood sample of a death case due to ARDS in a private hospital was collected from hospital laboratory. Father and mother of this patient were also sampled. Seven additional patients with ARI were also sampled. H5N1 was not detected in any of the 16 persons investigated. Testing of swabs is reported by the Influenza Group. Testing of sera and results are reported by the Avian Influenza Group.

Avian Influenza outbreak in Murshidabad and Dakshin Dinajpur, West Bengal

BV Tandale

Introduction

Avian influenza outbreak in poultry was reported in various districts of West Bengal in January 2008. Investigations were done in Murshidabad and Dakshin Dinajpur districts between 17th and 29th January 2008 as part of Central team. In a government poultry farm located in Balurghat headquarter of Dakshin Dinajpur district, 750 poultry bird deaths occurred between 6th and 10th January 2008 and H5N1 was confirmed in representative bird specimens. Culling operations were done between 16th and 21st January 2008.

Objective

- To undertake surveillance for detecting human infections in patients reporting acute respiratory infection following natural/occupational exposure

Work done

No suspect cases were detected in Murshidabad district during the period. Five infants (aged <1 year) hospitalized in Balurghat district hospital were suffering from fever, cough and respiratory distress. They had a history of poultry death in their backyard or in their neighborhood and were considered as “persons under investigation”. Throat swabs collected from these 5 infants (one also sampled with serum) were negative for H5N1 virus.

Two cullers reported fever, cough and respiratory distress after 1 and 5 days of culling. They reported past history of smoking since 10 years and were diagnosed with asthmatic bronchitis and chronic obstructive pulmonary disease respectively. Throat swabs and sera were collected from them. Throat swabs were negative for H5N1. Testing of swabs and sera are reported by Human and Avian Influenza Groups respectively.

Influenza

Influenza among school children in Panchgani, Satara district, Maharashtra

YK Gurav

Introduction

On 3rd March 2008, the authorities of New Era School in Panchgani, Satara (MS) reported increased incidence of fever, cough and cold among the school children.

Work done

Clinico-epidemiological investigations were done on 4th March 2008. Attack rate in residential New Era School was 42.4% (350/825). Cases were also reported from St. Peter's School and Sanjeewan Vidyalaya. Acute sera, throat swabs and urine samples were collected from 50 representative cases - 37 from New Era School, 7 from St. Peter's School and 6 from Sanjeewan Vidyalaya. Also, 3 community cases were sampled at a private clinic.

Influenza A (H3) was detected in throat swabs of 33 (62.3%) cases by RT PCR, the majority (29, 78.4%) were form New Era School. Influenza B was detected in 4 cases. Throat swabs of 4 cases from New Era High School yielded Influenza A (H3) isolates in MDCK cell line. Influenza A (H3) was identified as the major agent for the institutional outbreak. Additional details of testing are reported by Human Influenza Group.

Chikungunya

Chikungunya outbreaks in Satara and Kolhapur district, Maharashtra

BV Tandale

Introduction

Chikungunya outbreaks in Satara and Kolhapur districts were investigated on 3rd and 4th May 2007.

Work done

In Satara district, Nigdi village under Masur PHC reported CHIK-like illness outbreak on 20th April 2007. All 5 cases, 3 with convalescent phase sera and 2 with acute phase sera, were detected with anti-CHIKV IgM. In Kolhapur district, Hebbal village under Mungurwadi PHC, reported CHIK-like illness between 19th march and 5th April 2007. All 4 patients sampled with sera were detected with anti-CHIKV IgM. In Vaghrali village under Mungurwadi PHC, one case out of 2 sampled members of a family was positive for anti-CHIKV IgM. Also one of two health care workers was positive for anti-CHIKV IgM.

A fever outbreak, without joint manifestations, was reported in Bondivadi village under Adkur PHC in Kolhapur district between 9th and 17th April 2007. Two sampled cases were negative for anti-CHIKV and anti-DENV IgM. Entomological observations have been described by Entomology Group.

Chikungunya outbreaks in Kerala

BV Tandale

Introduction

State health authorities requested investigations for unusual presentations and/or higher attack rates in various districts of Kerala.

Work done

Between 13th and 19th June 2007, 33 hospitalized cases were investigated. One case admitted with hemorrhagic ascitis was positive for anti-DENV IgM antibodies in serum. CHIK-like illness was seen in 16 cases, of which 14 were detected with CHIKV etiology. Other 16 cases reported serious illnesses. Anti-CHIKV IgM antibody was detected in sera of cases with the clinical diagnoses like- exfoliative dermatitis, scrotal ulcerations, bullous rashes, recurrent urinary tract infections, urinary incontinence, altered renal function, myocarditis, meningoencephalitis, postviral Guillain Barre Syndrome, hepato-renal dysfunction (in 1 of 2 cases), hepatitis (in 1 of 2 cases) and hemorrhagic fever (in 1 of 3 cases).

Investigations were done on 89 patients from Malappuram and Kozhikode districts between 22nd and 29th October 2007. Entomological observations have been described by the Entomology Group. Chikungunya-like illness was seen in 82 patients, among which 69 were detected with CHIKV etiology. Other 7 patients had acute fever without joint pains. Among these 7 patients, 5 were detected with CHIKV etiology.

Chikungunya cases with neurological involvement, Pune, Maharashtra

BV Tandale

Introduction

During 2006 Chikungunya outbreak, CSF specimens with or without sera were referred by various hospitals for Chikungunya testing. We retrospectively evaluated these cases for documenting clinical course and outcome.

Work done

The clinical details and laboratory results noted in request forms were abstracted. Hospital chart record review of cases admitted in major hospitals in Pune was done. We tested these specimens for anti-DENV and anti-JEV IgM antibodies.

Request forms were available for 156 hospitalized suspect cases referred with CSF with or without sera. Clinical specimens included 123 pairs of CSF with acute phase sera and 33 CSF specimens. Anti-JEV IgM antibodies were detected in 6 cases. Anti-DENV and anti- JEV IgM antibodies were detected in serum of 1 case. Another 7 cases had Chikungunya with evidence of anti-DENV/JEV IgM antibodies. Ninety-seven (62.3%) cases were confirmed as Chikungunya- Anti-CHIKV IgM antibodies in sera of 20 cases, in CSF of 21 cases, in serum-CSF pairs of 54 cases, CHIKV RNA in CSF and serum of a case each. Majority were from Maharashtra (80, 82.5%), followed by Karnataka (15, 15.5%) and a case each from Madhya Pradesh and Gujarat. Majority were males (59, 60.8%) and

above 60 years of age (36, 37.1%). One (13, 44.8%) or more (16, 55.2%) comorbidity was seen in 29 (29.9%) cases.

Hospital chart record review was done for 92 cases, which included 58 cases confirmed as Chikungunya. Neurological involvement was seen exclusively in 24 (41.4%) of 58 cases. Multi-systemic involvements noted in 34 (58.6%) of 58 cases were renal (22), respiratory (11), hemorrhagic (8), cardiac (8), hepatic (7) and electrolytemic (6). Eight cases died, all were males, 5 were above 60 years and comorbidity was seen in 5 of them. Age >60 years (Chi-square of 4.70, df=1, $p< 0.05$; OR of 1.67) and comorbidity (Chi-square of 6.43, df=1, $p< 0.05$; OR of 4.45) were detected as the significant risk factors for multi-systemic involvements, but not for deaths.

Dengue

Dengue outbreak in Jabalpur, Madhya Pradesh

BV Tandale

Introduction

An outbreak of Dengue-like illness was reported during September 2007 in Jabalpur and adjoining districts like Satna, Narsingpur, Rewa, Damoh and Karwa.

Work done

Cases were identified and sampled from major hospitals in Jabalpur. A planned serosurvey in Barela, an urban locality 20 kms away from Jabalpur city, was done and 298 sera were collected. Verbal autopsies of 21 deaths reported during the period were also done. A house-to-house survey was done with the help of medical students for estimating the attack rates in the community. Entomological observations are described by the Entomology Group.

Among 247 patients with sera, 70 were confirmed as Dengue, which included 2 cases detected with DEN-2 viral RNA. Among 298 subjects surveyed in Barela, 18 of 140 symptomatic patients were detected with anti-DENV IgM antibodies. The features of Dengue-like illness were noted in 16 of 21 deaths on verbal autopsy. The attack rates in the community averaged at 20 to 30%. Dengue-2 serotype was identified as the etiological agent of the outbreak.

Chandipura

Chandipura Viral Encephalitis in Children in Nagpur division (MS)

BV Tandale

Introduction

An outbreak of acute viral encephalitis in children, reported in Nagpur division by the State Health Department, was investigated between July 12 and September 13, 2007.

Work done

In addition to investigations of hospitalized cases, follow up examination in the field for neurological sequelae in recovered cases with collection of convalescent phase sera, and a planned serosurvey focusing on children <15 years of age were done. The State Health Department has line-listed 55 patients with 32 deaths (CFR 58.2%). Male to female ratio was 1:1.1. Age distribution showed preponderance in <5 years of age. Forty-seven cases had features of Acute Encephalitis Syndrome (AES). The clinical specimens included 6 CSF, 50 acute sera, 22 early

convalescent-phase sera, and 2 late convalescent-phase sera collected from 48 patients. All specimens were tested for anti-JEV IgM, anti-CHPV IgM/IgG and CHPV RNA.

All samples were negative for anti-JEV IgM. There were 3 CHPV isolations from acute phase sera. Two sera - CSF pairs were positive for CHPV RNA. Additional 23 acute-phase sera showed presence of CHPV RNA. Seroconversion was detected in 8 patients. Also, anti-CHPV IgM antibody was detected in 5 patients. Thus, 31 patients had CHPV evidence.

Additional 85 non-line-listed cases with 16 CSF, 79 acute sera and 13 convalescent sera were investigated. All were negative for anti-JEV IgM antibodies. One acute-phase serum showed presence of CHPV RNA and seroconverted to anti CHPV IgG. Seroconversion was also detected in 4 other cases. Anti-CHPV IgM was detected in 4 cases. Thus, 9 cases had CHPV etiology.

Serosurvey was carried out in 33 (16 affected and 17 unaffected) localities of 6 districts in Nagpur Division. A total of 542 sera were collected. Anti CHPV IgM, IgG and both IgM and IgG antibodies were detected in 30 (5.5%), 82 (15.1%) and 5 (0.9%) children respectively. Fever was reported in 9 (30%) of 30 detected with anti CHPV IgM, as against 14 (17.1%) of 82 detected with anti CHPV IgG antibodies.

Chandipura & Japanese encephalitis in adults in Barsi, Solapur, Maharashtra

YK Gurav

Introduction

Sera of three AES cases were referred for viral diagnosis. Chandipura and JE IgM detection in these sera led to the verification by the field investigations.

Work done

Acute sera of two adult male cases, resident of Kusalamb and Kalegaon in Barsi, diagnosed as viral encephalitis were detected with anti-CHPV IgM antibodies. These sera were negative for anti-JEV and anti-DENV IgM antibodies. Verbal autopsy of these patients suggested Acute Encephalitis Syndrome (AES). From these villages, 31 and 25 contact sera were collected respectively. One male patient aged 72 years from Kalegaon had fever 1 month back and was detected with anti-CHPV IgM and IgG antibodies. In addition, 5 patients each from Kusalamb and Kalegaon were detected with anti-CHPV IgG antibodies. Additional two patient deaths from Khandvi village suggested AES on verbal autopsy. Among 11 contacts sampled from Khandvi, serum of a male aged 15 years with history of fever for 3 days was detected with anti-CHPV IgM antibody.

A patient residing in Surdi, recovered with AES, was detected with anti-JEV IgM antibody in sera collected on 12th and 55th day of illness. Among 33 contact sera collected from Surdi, a male patient aged 5 years with fever for 3 days was detected with anti-JEV IgM antibodies. Other four patients having fever more than 3 days were detected with anti-CHPV IgG antibodies. Chandipura and JE in adults in non-endemic area are detected for the first time. Entomological observations have been described in Entomology Group.

Childhood Encephalitis in Warangal, Andhra Pradesh

BV Tandale

Introduction

Acute viral encephalitis cases in children were being admitted in pediatric wards of MGM Hospital, Warangal.

Work done

Acute phase sera of 10 cases were collected in June 2007, 8 of them were detected with CHPV RNA by PCR. One of the RNA positive serum yielded CHPV isolate. Additional 6 CSF and 79 sera from VBRI, Hyderabad, and 2 CSF and 19 sera from Niloufer Hospital, Hyderabad were also investigated. A contact serosurvey was carried out, in which 11 convalescent sera and 132 contact sera were collected.

Three of 6 CSF and 4 of 79 sera from VBRI were detected with anti- CHPV IgM antibodies. Also, 3 sera were positive for anti-JEV IgM antibodies. Among 14 cases from Niloufer Hospital, 2 were detected with anti-CHPV IgM antibodies. One case seroconverted to anti-CHPV IgM antibodies in convalescent-phase sera. Among 132 contact sera, one showed anti-CHPV IgM antibodies.

Serological survey of humans in Warangal and Adilabad districts of Andhra Pradesh for Japanese Encephalitis Virus

BV Tandale

Introduction

A pilot study in a severely affected district i.e. Warangal and less affected district i.e. Adilabad was done in June 2005. Analysis of sera for CHPV, JEV and WNV neutralizing antibodies was reported earlier. Neutralizing (N) antibodies against JEV and WNV were tested by JE Group.

Work done

Testing of 391 sera out of 409 sera collected during serosurvey in Warangal district was done. Seropositivity to N antibodies was detected in 21 (5.37%) against JEV, in 90 (23.02%) against WNV and in 194 (49.62%) against both JEV and WNV. Seropositivity of N antibodies against both JEV and WNV in rural areas was (156/341, 45.75%). Seropositivity in >15 years age group (168/299, 56.19%) was significantly higher than in <15 years age group (26/92, 28.26%), indicating the increasing seropositivity with age. Seropositivity in males (88/166, 53.01%) was significantly higher than in females (68/225, 30.22%).

Publications

- Tandale BV, Tikute SS, Arankalle VA, Sathe PS, Joshi MV, Ranadive SN et al. Chandipura virus: A major cause of Acute Encephalitis in children in North Telangana, Andhra Pradesh, India. **J Med Virol** 2008; 80(1): 118-124.

Workshops / Conferences / Seminar / Meetings attended

BV Tandale

- Participated in Systematic reviews workshop organized by Cochrane Group at CMC Vellore on 25th and 27th June 2007.

- Invited lecture on “Chikungunya - Epidemiological and clinical aspects” in International conference of viral diseases in tropics and sub tropics, organized by Indian Virological Society at IARI, New Delhi between 11th and 14th December 2007.
- Presentation on “NIV experience” in National Workshop on emerging fevers: Chikungunya organized by NRHM Kerala, Kochi on 28th December 2007.
- Presentation on “The role of virology laboratory” in workshop on Pandemic influenza preparedness plan organized by the Director of Health Services, Government of Maharashtra, at Mumbai on 5th March 2008.

YK Gurav

- Poster presentation on “Chandipura viral encephalitis in children in Nagpur division, Maharashtra” in International conference of viral diseases in tropics and sub tropics, organized by Indian virological Society at IARI, New Delhi between 11-14 December 2007.
- SPSS 15.0, Training organized by SPSS South Asia and School of Management Studies, Jawaharlal Nehru Technological University, Hyderabad during 7-9 September 2007.

Viral Hepatitis

Viral Hepatitis

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2 Viral Hepatitis

- Development of candidate vaccine for hepatitis E
- Development of combined vaccines for Hepatitis B and E viruses
- Generation of infectious cDNA clones for swine and human HEV and chimeric swine-human HEV clones
- Fulminant Hepatitis E: Association with cytokine polymorphisms and viral sequence variations
- Study of processing and characterization of ORF1 encoded protein/s of Hepatitis E virus
- Detection and characterization of Hepatitis E virus from pig liver tissue sold in local markets
- Virological analysis of water and water / sewage treatment plants with special reference to Hepatitis A and E viruses
- Detection of Hepatitis A and E viruses in soil samples
- Genomic characterization of Hepatitis E and A viruses in India for 28 years
- Genomic analysis of Hepatitis A virus isolates from different geographic locations of India
- Therapy in patients with chronic hepatitis C: A randomized control trial of interferon with ribavirin and combination of interferon with Glycyrrhizin
- Evaluation of immunogenicity of recombinant hypervariable region and non-structural region 3 of Hepatitis C virus as vaccine candidates
- Hepatitis B surface antigen-specific T cell memory in individuals who had lost protective antibodies after Hepatitis B vaccination
- A multicentric randomized controlled clinical trial of Adefovir, Adefovir+Lamivudin and combination of Adefovir and Glycyrrhizin in HBV related decompensated cirrhossis

Development of candidate vaccine for hepatitis E

VA Arankalle, KS Lole, TM Deshmukh.

Hepatitis E is an important public health problem in India with several epidemics being reported all over the country causing morbidity and mortality (particularly in pregnant women). In sporadic settings, fulminant hepatitis E has been observed in men and non-pregnant women. Travellers to endemic areas, military personnel, elderly individuals, sewage workers etc. are at a high-risk of HEV infection. Therefore, there is a need for hepatitis E vaccine.

Objectives

- To develop recombinant protein and/ or DNA based vaccine for hepatitis E.

Work Done

As described earlier with our earlier experimental work in mice and rhesus monkeys NE region (458 aa- 607 aa, neutralizing epitope) within HEV ORF2 emerged as a promising vaccine candidate. NE was found to be effective as combination of protein plus DNA in eliciting excellent antibody titers and showed better protection in challenged monkeys as compared to complete ORF2-based vaccine candidates. We used nickel affinity column purified NE protein of about 80% purity level for these studies. Further purification of NE protein was done using gel filtration column chromatography (Sephacryl 100 column, AKTA BASIC 100 System) and purified protein fractions were analysed on native and denaturing PAGE. Two separate peaks of NE protein were obtained after gel filtration and on characterization they were found to be aggregates of NE protein. These peaks are being evaluated separately as immunogens in mice.

Immunogenicity of NE (HEV genotype 1 & 4) in mice

To understand the role of DNA and protein components of the vaccine and to assess the utility of genotype 4 NE protein/ DNA as vaccine, pVAX1 constructs and E.coli expressed NE proteins of both the genotypes were evaluated as either protein alone or in combination with DNA in mice. Twelve groups of 6-8 weeks old female Swiss albino mice (n=10/ group) were immunized with 1µg/dose of pVAX1+NE (HEV genotype 1/4) plasmid DNA construct. Total 3 doses were given, first 2 by gene gun (DNA) and the last one (protein) intramuscularly (50µl in each hind leg muscles) at 0, 4 and 8 weeks interval. The mice were bled before immunization. Genotype 4 NE protein was found to be less immunogenic as compared to genotype 1 NE. With our earlier studies, complete ORF2 proteins of genotype 1 (human) and genotype 4 (swine) HEV were found to be equally efficient in detecting anti-HEV antibodies due to type 1 or type 4 infections in ELISA.

Future plans

To determine immunogenic regions within the swine ORF2 using truncated genotype 4 ORF2 constructs.

Development of combined vaccines for Hepatitis B and E viruses.

Shubham Srivastava, KS Lole, AS Tripathy, VA Arankalle

The aim of this project is to develop combined vaccine for Hepatitis E and Hepatitis B. Our earlier results documented that NE region of HEV ORF2 and 'S' gene of HBV in liposome formulations as combinations of

respective proteins plus DNAs represent excellent immunogens and induce enhanced and early antibody responses (2 weeks post 1st dose) as compared to the respective single antigens given separately. Cellular immune responses were further analysed by assessing the cytokine levels, Th1/ Th2 inclination and memory T cell responses with respect to both the antigen components.

Work done

Swiss albino mice were immunized with different liposome formulations as mentioned in **Table 1** and tested for antibody as well as cellular responses by specific ELISAs and lymphocyte proliferation assays respectively using NE antigen (NEAg) and HBsAg as the recall antigens.

Table- 1: Antibody and cellular responses in mice immunized with different formulation

Liposome formulations	% Sero-conversion 2 weeks post third dose	No. of Responders /total mice tested (recall antigen)
pVAX1	0	0/8 (NEAg and HBsAg both)
HBV DNA	0	Not done
HBV Protein	100	7/8 (HBsAg)
NE DNA	0	Not Done
NE Protein	100	5/8 (NEAg)
HBV DNA + NE DNA	0	2/8 (HBsAg) 4/8 (NEAg)
HBV Protein + NE Protein	100 (anti-HBs) 100 (anti-HEV)	Not Done
HBV DNA + HBV Protein + NE DNA + NE Protein	90 (anti-HBs) 100 (anti-HEV)	Not Done
HBV DNA + HBV Protein	100	7/8 (HBsAg)
NE DNA + NE Protein	100	7/8 (NEAg)
HBV Protein + pVAX1	100	6/7 (HBsAg)

Antibody isotype analysis

In NE immunized mice groups, IgG1 was the pre-dominant subtype of IgG irrespective of the formulations. In HBV immunized mice, protein alone and DNA + protein formulations showed predominance of IgG1 and IgG2a isotypes respectively. When protein alone combination was used IgG1 isotype was predominant against both HBsAg and NEAg components of the vaccine whereas with DNA plus protein combination formulation, balanced IgG1 and IgG2a levels were noted against both the antigens studied. (**Fig. 1 & 2**).

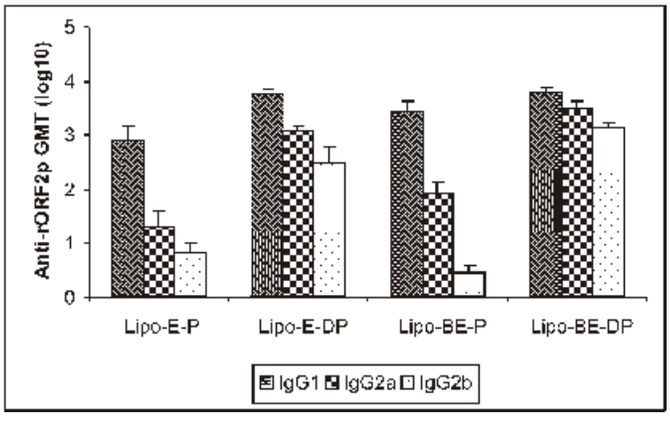


Fig.1 : Anti-HEV (ORF2) IgG subtype analysis in mice immunized with different liposome formulations

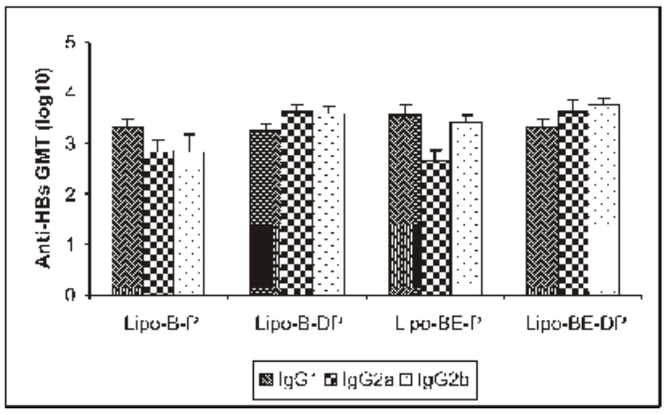


Fig. 2 : Anti-HBs IgG subtype analysis in mice immunized with different liposome formulations

Future plans

Promising vaccine candidates will be evaluated in rhesus monkeys.

Generation of infectious cDNA clones for swine and human HEV and chimeric swine-human HEV clones

KS Lole, VA Arankalle

Understanding of basic biology of HEV, mechanism of its pathogenesis, virus replication strategies have suffered on account of lack of efficient cell culture system and practical animal model. In order to know viral genes responsible for species specificity and pathogenicity of HEV we are developing type 1 and type 4 chimeric viruses. Genotype-1 HEV was successfully cloned in the vector pGEMT EASY ((pT1FGPM2K) in the previous year. There were total 50 nucleotide changes in clone pT1FGPM2K as compared to the original full-genome sequence of the virus.

Work done

In vitro transcripts of the clone were used to evaluate its replication competence in different cell-lines [human hepatoma cell lines (HepG2, Huh-7 and PLC-PRF/5) and nonhepatic cell lines (RD, human rhabdomyosarcoma and Vero E6, African green monkey kidney epithelium)]. Viral antigens were detected by immunofluroscence assay (IFA).

All the three human hepatoma cell lines showed positively stained cells suggesting that the transfected viral genome may have replicated in the liver-derived cells. The positivity was detected from 3 days post transfection onwards up to 10 days in three cell lines. There were no obvious cytopathic effects seen after transfection however PLC- PRF/5 cells started floating 8 days post transfection and came off completely by 10 days. Overall, IFA positivity of the cells was between 10-15%. All the hepatoma cells showed increase in the percent IFA positivity from 4 days post transfection to 10 days. The transfected RD and Vero E6 cells were also positive in IFA but their positivity remained same from 4 days posttransfection upto 10 days. Viral RNA copies in the cells and supernatants were quantitated using Taqman real-time reverse transcription-PCR assay. Virus could successfully egress from PLC-PRF/5 cells and supernatant had 10 folds more virus in the 6th day supernatant than that in the cell pellets. The other two hepatoma cells (HepG2 and Huh-7) showed comparatively less virus release. Similarly, the two non-hepatoma cells, RD and Vero E6 were not able to support virus egress to the same extent as that by PLC-PRF/5. Thus, so far, PLC-PRF/5. cells appear to be yielding maximum yield of the virus.

Future plans

- Development of genotype 4 HEV infectious cDNA clone
- Construction of type1/ type4 chimeras

Fulminant Hepatitis E: Association with cytokine polymorphisms and viral sequence variations

Nischay Mishra, VA Arankalle

Hepatitis E present in both epidemic and sporadic forms is an important public health problem. Infection with HEV can be asymptomatic, self-limiting acute hepatitis or fulminant hepatitis. Pregnant women constitute a high-risk group for fulminant hepatic failure (FHF) with high mortality. We have been trying to understand pathogenesis of HEV infection. Earlier, association of Th1 and Th2 cytokines, viral load and antibody titres were evaluated in different clinical conditions. Based on the results obtained, further studies on TNF-α, IFN-γ and IL10 were considered necessary. In addition, evaluation of relationship of mutations in the viral genome with FHF needs to be explored

Objectives

- Identification of specific mutations, if any, in viruses recovered from FHF patients when compared with self-limiting hepatitis E patients-derived sequences at full genome level.
- Association of cytokine polymorphism(s) with susceptibility / severity in HEV infection.

Work done

Two hundred samples were obtained during the epidemic of hepatitis E at Puntamba, PBMCs were isolated and stored in -70^{O}C. for further cytokine polymorphism studies. Cytokine polymorphism assays for IL-6 and IL-10 were standardized. Full genome sequencing was done from the serum of one FHF-E sample. With this, 5 full genomes from FHF-E cases have been sequenced. Three isolates from Lonavala epidemic (year 2000) were amplified and sequenced at full genome level. Phylogenetic analysis showed that all the three genomes belonged to the same cluster and were close to Nepal isolate.

Future plans

- To assess whether single nucleotide polymorphisms are associated with susceptibility or severity in hepatitis E infection.
- Sequence analyses for genomes obtained from Fulminant and acute-resolving hepatitis E patients.

Study of processing and characterization of ORF1 encoded protein/s of Hepatitis E virus

Yogesh Karpe, K S Lole

ORF1 of HEV is known to encode for viral nonstructural polyprotein with four putative domains indicative of methyltransferase (MeT), papain-like cysteine protease (PCP), RNA Helicase (Hel), and RNA dependent RNA polymerase (RdRp).

Objective

- To study the processing of ORF1 protein.

Work done

In order to characterize helicase domain, this protein domain was expressed in E.coli with His- tag and purified first using Ni-resin column and then by ion exchange chromatography. Purified protein was confirmed by western blot staining with anti-His antibodies. To check for the binding and unwinding properties of helicase, ^{32}P end labeled partially double stranded RNA and DNA molecules with 3' and 5' overhangs were incubated separately with the enzyme and products were analysed by acrylamide gel electrophoresis and autoradiography. Helicase showed unwinding of molecules with 5' overhangs.

Subgenomic constructs from full-length cDNA clone of HEV (pT1FGPM2K) are being developed by replacing structural protein region (ORE2) by reporter gene. For that, neomycin resistance gene (794 bp) was amplified from vector pcDNA3.1 and similarly GFP gene (716bp) was amplified from vector pAcGFP1N1. By using a primer pair specific to the 5' and 3' ends of the gene including a 3'-terminal HindIII restriction site. The 5' end of the neo gene was extended with 4006-5202 fragment of pT1FGPM2K by fusion PCR, including a 5'-terminal SfiI restriction site. The resulting fused PCR product was digested with SfiI and HindIII and substituted into pT1FGPM2K to yield pT1FGPM2K-2Neo and pT1FGPM2K-2GFP respectively. These constructs were sequenced completely and found to be intact.

Future plans

- Use of HEV subgenomic constructs to find out the role of different regions of ORF1 in HEV replication.
- Characterization of helicase enzyme activity.

Detection and characterization of Hepatitis E virus from pig liver tissue sold in local markets

VA Arankalle, MA Kulkarni

Hepatitis E Virus (HEV) is a major causative agent responsible for sporadic and epidemic acute viral hepatitis in developing countries. There is ample evidence for zoonotic transmission of this virus. Past evidence shows that in Indian scenario genotype 1 is prevalent in humans where as genotype 4 is seen in swine. In Japan, cases of acute hepatitis E were linked to consumption of undercooked pig liver and deer meat. Data on the presence of HEV in animal meat is lacking from India.

Objective

To detect and characterize Hepatitis E Virus from pig livers sold in local markets.

Work done

A total of 240 pig liver samples were purchased from local markets. Total RNA was extracted from 100 mg of liver tissue using TRIZOL reagent. Samples were screened for HEV RNA employing both degenerate primers and genotype 4 specific primers from the ORF2 region. Of the 240 pig liver samples screened by nested RT-PCR, two (0.83%) were found to be positive for HEV RNA. Further, HEV RNA positive samples were confirmed using ORF1 region specific primers. Phylogenetic analyses based on ORF1 region (4641 to 5062 nt) and ORF2 region

sequences placed both sequences in genotype 4. These Indian swine HEV ORF1 and ORF2 region sequences, INDSW-07-J1, INDSW-07-P1, INDSW-07-J2, and INDSW-07-P2 respectively showed 92 % identity to each other. INDSW-07-J1, INDSW-07-J2, INDSW-07-P1, INDSW-07-P2 sequences showed 91% similarity with previously reported Indian swine HEV strain from Pune collected in the year 2000, IND-SW-00-01, while the sequences, INDSW-07-P1 and INDSW-07-P2, showed 90 % and 91% identities with the northern and western Indian isolates.

Virological analysis of water and water / sewage treatment plants with special reference to Hepatitis A and E viruses

Vikram Verma, VA Arankalle

The study of viruses in water and wastewater is one of the important branches of natural sciences. Water borne diseases are spread by contamination of drinking water with urine and faeces of infected animals and/ or people. Contamination of water systems may occur due to floodwaters, water runoff from landfills, septic fields and sewage pipes. Presence of pathogenic hepatitis viruses and enteric viruses has been reported from water bodies throughout the world. No systematic data on the prevalence of these viruses in drinking water has been reported so far from India.

Objectives

- To evaluate water supplied by Pune municipal corporation for the presence of various disease causing waterborne viruses during one year
- To assess the efficiency of domestic water purification systems in eliminating viruses employing HEV as model virus.
- To generate data on prevalence of enteric viruses (HAV, HEV, enterovirus and Rotavirus) in Mutha river

Work done

Standardization of multiplex PCR

Multiplex PCR for simultaneous detection of HAV, HEV, enterovirus and rotavirus was standardized with a new set of primers for rotaviruses NSP5 gene. Further sensitivity of multiplex PCR was compared with single PCR for each virus in limiting dilution experiments **(Fig. 3)**.

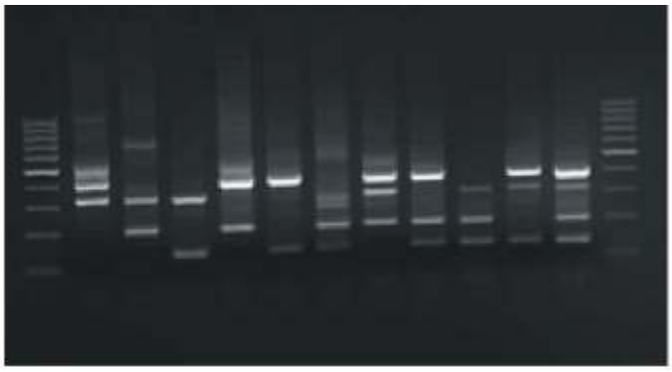


Fig. 3: Standardization of multiplex PCR

Collection of drinking water samples

- Drinking water samples were collected from three water treatment plants (Parvati, Warje and Lashkar) and from some points of common public use.
- Fifteen samples were collected weekly, 6 from treatment plant and 9 from points of common public use. A total of 662 samples were collected.
- Forty liter of water/sample was collected and concentrated up to 4.0 ml.
- Concentrated water samples were subjected to nested multiplex PCR for detection of HAV, HEV, Enteroviruses and Rotavirus

Collection of river water samples

A total of 64 river water samples were collected from four different points along Mutha River

Testing of the samples

Out of 536 drinking water samples screened so far by nested PCR, 2 samples were positive for enterovirus (5'NCR primers).

River water samples were subjected to multiplex nested PCR for the detection of HAV, HEV, Entero and Rota viruses. A representative number of samples (n=10) were subjected to individual nested PCR for detection of HAV, HEV, Enteroviruses and Rotavirus to verify the sensitivity of multiplex PCR. The results of individual nested PCR were comparable with those with multiplex nested PCR.

Table-2 : Viral RNA positivity by PCR

Virus	Positivity in PCR/ total samples tested
HAV.	49/64
HEV	16/64
Enterovirus.	33/64
Rotavirus.	36/64

Detection of Hepatitis A and E viruses in soil samples

Deepti Parashar, VA Arankalle

Hepatitis A and E are enterically transmitted viral diseases of global public health importance. Faecal -oral route is the predominant transmission mode leading to common source outbreaks as a result of consumption of contaminated food and water. Contamination of water supplies with sewage is the commonest observation. Data on the presence of hepatitis A and E virus in soil samples is not available.

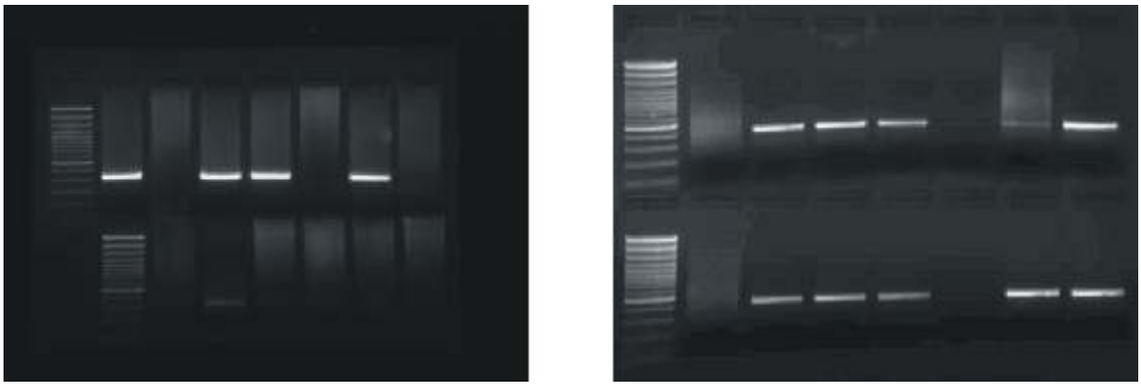
Objectives

- Detection of Hepatitis A and E virus in soil samples

Work done

For the detection of Hepatitis A and E viruses in soil, we tried a number of RNA extraction methods. The CTB extraction method with slight modifications was found to yield optimum results.

A total of 150 soil samples were collected from four different points along Mutha River, Pune and 3 samples were collected from Pashan lake. Out of 150 soil samples 54 were positive for HAV and 14 were positive for HEV by nested PCR. Pashan lake samples were negative for HAV and HEV **(Fig. 4)**.



(a) (b)

Fig. 4: Reverse-transcription PCR products of a) HAV and b) HEV

Genomic characterization of Hepatitis E and A viruses in India for 28 years

VA Arankalle, MA Kulkarni, LP Chobe

This project was sanctioned by the ICMR under 'Genomics' category.

Objectives

- To assess mutation rates of Hepatitis A and E Viruses recovered during 1979-2007.
- To evaluate the role of genotype 4 HEV shown to circulate in Indian pigs in causing human disease.
- Genomic characterization of swine HEV isolates from western and southern India

Work done

Molecular surveillance

During this year, 95 hepatitis A and 10 hepatitis E cases clinically diagnosed and serologically confirmed on the basis of the presence of IgM anti-HAV and IgM anti-HEV antibodies were investigated. Out of 95 hepatitis A samples tested, 86 were HAV RNA positive. Similarly, out of 10 acute samples screened for HEV RNA, only one was positive. All PCR positive samples were processed for sequencing. Based on the phylogenetic analysis, all HAV and HEV samples were classified as genotype III A and genotype 1 respectively.

Full Genome sequencing of HAV strains from sporadic cases from western India: (1995-2007)

To assess molecular evolution of HAV, full-length HAV genome sequencing was undertaken. Three sporadic anti-HAV IgM positive serum samples representing years 1995, 2006 and 2007 from Pune were amplified and sequenced. Stored samples from earlier years (1981-1994) did not lead to amplification of most of the genome and therefore could not be used for this study. Sequence alignments were generated using Mega-3.1 version. The genomic length of HAV from sample 2006 was 7460 nt, sample 2007 was 7461 nt and sample 1995 was 7459 nt excluding poly A tract at 3' terminus. The three HAV isolates showed 97-98 % identity with each other, differed from each other only by 2-3% in the entire genome. Comparison of the three HAV isolates against all the reported HAV full genomes revealed that HAV from sample 2007, sample 2006 and sample 1995 are 96.8%, 96.7% and 98%

respectively, related to the German IIIA strain AY644337 respectively. Thus, indicating that all the three HAV full genome sequences from India belonged to subgenotype IIIA. The sequences of HAV from samples 2006, 2007 and 1995 were only 80.3 to 83% identical with other reported human HAV genomes of Genotype I and II and simian isolate of genotype V. The amino acid sequences of HAV from sample 2006, sample 2007 and sample 1995 showed changes in 6, 11 and 5 amino acids as compared to prototype strain AB 279732 (Japan).

Last year, we had reported results of the investigations of an outbreak hepatitis A at Shimla, Himachal Pradesh reported during January-February 2007. Based on partial genome sequencing, the virus was classified as genotype IIIA. Since this represented the first major outbreak of HAV in north India, one of the RNA positive serum sample (SIM27) was further processed for full-genome sequence analysis. The genomic length of SIM27 isolate was 7450 nucleotides. It possessed a single long ORF of 6711 nucleotides encoding the polyprotein of 2237 aa with 3'UTR of 39 nucleotides. A representative number (2/7) of sequences from Shimla outbreak are shown in the phylogenetic tree as all 7 RT-PCR positive samples were 100% homologous in the 5'NCR .

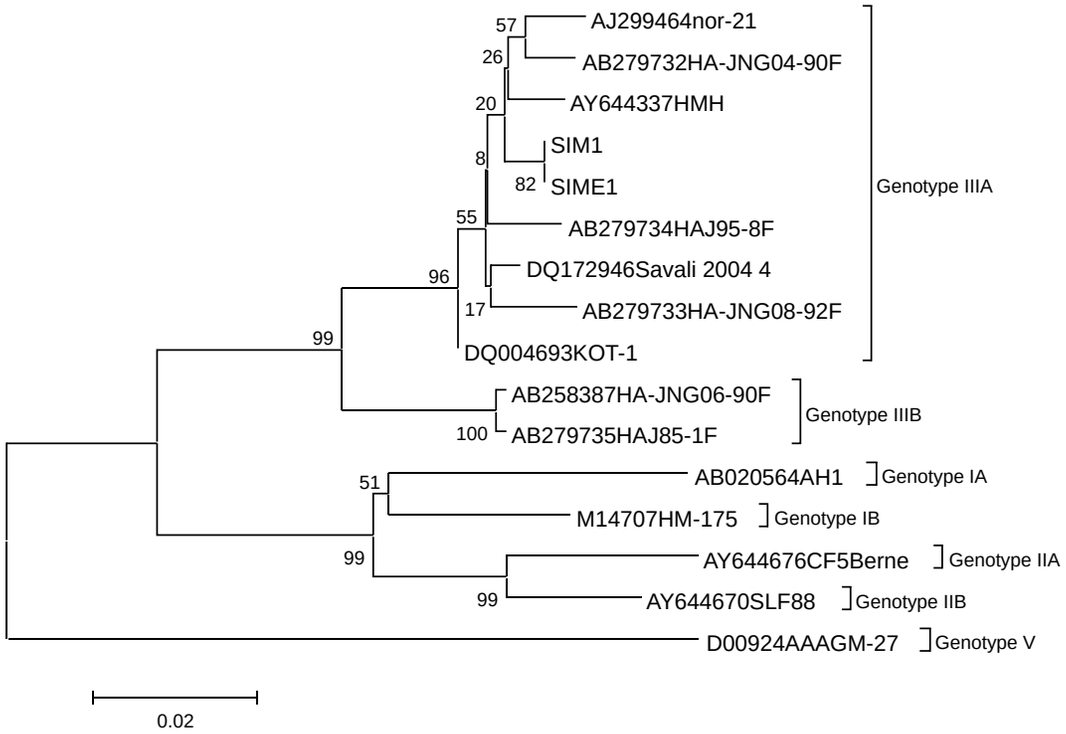


Fig. 5. Phylogenetic tree based on 5'non-coding region

Genomic analysis of Hepatitis A virus isolates from different geographic locations of India

SD Chitambar, MS Joshi, Shilpa Bhalla

HAV infection in India has been highly endemic, however, its current status presents the features of both developing and developed countries. Increase in the clinical disease burden in adults is suggestive of shift from high to intermediate endemicity of hepatitis A. HAV causes fulminant hepatic failure and also exists with hepatitis B and E infections. In view of this, monitoring of HAV genotypes and strain variations have been considered of importance.

Objective

- To characterize wild type hepatitis A virus strains recovered from different parts of India using 5'NCR, VP1/2A junction region and partial RNA polymerase region.

Work done

To determine the HAV genotypes prevailing in India, stool and serum samples were collected from hepatitis A patients during 2004-07 from western (n=12), southern (n=15) and northeast (n=4) parts of the country. Genomic analysis using VP1/2A junction region indicated genotype IIIA as prevailing genotype. Analysis of partial RNA polymerase region showed clustering of Indian strains with HAV strains from Norway and Germany. Nor21 strain of sub genotype IIIA was used as the reference strain. Percent nucleotide identities with Nor21 (genotype IIIA) ranged from 95.60 -99.0 in 5'NCR, 95.0-97.90 in VP1/2A junction and 92.90 -98.20 in partial 3D regions for western region. In southern region it ranged from 97.90 -99.0 in 5'NCR, 95.0-98.0 in VP1/2A junction and 94.60 -98.20 in partial 3D regions while in northeast region it ranged from 98.0 -98.50 in 5'NCR, 95.40-97.40 in VP1/2A junction and 95.50 - 97.30 in partial 3D regions.

Variations between the strains from western, southern and northeast regions were detected to be 1.2% - 1.4% in partial 5'NCR while the same were 2.2% - 2.5% in VP1/2A junction and 1.8% - 3% in partial 3D regions.

Therapy in patients with chronic hepatitis C: A randomized control trial of interferon with ribavirin and combination of interferon with Glycyrrhizin

VA Arankalle

The ICMR is conducting two multicentric drug trials entitled “A randomized control trial of interferon with Ribavirin and combination of Interferon with Glycyrrhizin" and “Therapy in patients with chronic hepatitis C and HCV induced cirrhosis”. NIV is the coordinating laboratory for Virology.

Work done

During the current year, a total of 72 samples were tested for HCV RNA and 39 were scored HCV RNA positive. HCV RNA quantitation was carried out for 63 samples using Amplicor HCV Monitor test version 2.0 (Roche Diagnostics) Kit. Core gene sequence-based HCV genotyping was carried for 10 samples. Based on the phylogenetic analysis, the distribution of genotypes was 5 (3a), 2 (3b), 2 (1b) and 1(3i).

In order to assess the influence of HVR1 variability on the response to interferon therapy, day “0” samples of 48 patients from the first trial were processed this year. All the 48 samples were PCR amplified and cloned using pGEMT Easy vector. For each sample 30 HVR clones were sequenced for the analysis of quasispecies. Similarly, 27 samples representing 17 patients (either non-responders or transient responders) collected at different time points were also processed. Sequence variation with respect to response to interferon therapy is being analysed.

Evaluation of immunogenicity of recombinant hypervariable region and non-structural region 3 of Hepatitis C virus as vaccine candidates

Gauri Gupte & VA Arankalle

Hepatitis C virus, a major causative agent of chronic hepatitis affects more than 170 million individuals worldwide, and can evolve towards cirrhosis and hepatocellular carcinoma. Therapeutic treatments consisting of pegylated interferon alpha and ribavirin are effective in less than 50-80% of cases and are associated with severe side effects. Various attempts for the development of vaccine have been made. However, no efficacious vaccine has been developed so far. Hence, development of an effective vaccine becomes imperative. Genotype 3 is most common in

India. Most candidate vaccines are being developed employing genotype 1 strains prevalent in the developed countries. It is therefore obligatory for us to concentrate on genotype 3, especially considering the genetic heterogeneity of HCV between and within genotypes and in the same individual.

Objective

- To evaluate immunogenicity of recombinant Hypervariable Region 1 (HVR1) and Non-structural region-3 (NS3) of genotype 3 Hepatitis C virus as possible candidate vaccines either as DNA or peptide polytope or as a combination of both.

Work done

Very little sequence information is available for HCV genotype-3. As HVR1 is one of the important targets for us to develop the vaccine, we amplified this region for genotype-3 samples. These included: 3a (28), 3b (6), 3e (2), 3f (1), 3g (9) and 3i (2). The PCR products were cloned using pGEMT Easy vector and 30 HVR clones were screened per patient. This information is being analysed using bioinformatic approach to obtain consensus sequence.

Hepatitis B surface antigen-specific T cell memory in individuals who had lost protective antibodies after Hepatitis B vaccination

AS Tripathy, VA Arankalle

Hepatitis B vaccination in a normal population is associated with a non-responder rate of 5%. Long-term protection after Hepatitis B vaccination is dependent on the persistence of a strong immunologic memory. Loss of anti-HBs may be associated with the risk of hepatitis B. However, more recent studies indicate that immune memory persists beyond declining of anti-HBs levels below the detection limits and protects against infection. In case of HBV exposure, the immune memory rapidly leads to a vigorous anamnestic response, which prevents acute infection.

Objective

- To detect and characterize HBsAg specific T cell reactivity in the hepatitis B vaccinees.

Work done

The T cell population that is supposed to contain the specific memory cells (CD4+/CD45R0) T cells was isolated and analysed for HBsAg-specific IFN-gamma secretion by enzyme-linked immunospot assays (Elispot). The vaccinees were grouped in two categories (1) anti-HBs positive and T cell responders as assessed by LPA (n=17) and (2). anti-HBs negative (negativity over time) and T cell responders (n=8). In the first category, 10/17 and 6/8 vaccinees from the two categories had significantly high IFN-gamma (representative cytokine for the Th1 subset of CD4 T cells) secreting memory T cells. This observation is in good agreement with earlier data pointing to a dominant Th1 cell response after Hepatitis B vaccination and existence of functional memory T cells in a group of individuals who had lost anti-HBs over time after the vaccination.

Future plans

Studies will be continued by increasing the sample size.

A multicentric randomized controlled clinical trial of Adefovir, Adefovir + Lamivudin and combination of Adefovir and Glycyrrhizin in HBV related decompensated cirrhossis

VA Arankalle

ICMR is conducting a Task force, multicentric project mentioned above with NIV as the coordinating laboratory for Virology. The project aims at comparison of efficacies of different drug combinations in treating HBV related decompensated cirrhosis, a major serious problem in India.

Objective

In addition to the primary objective of comparing different drug regimes, the aim of the study is to study the role of HBV genotypes, viral load and mutations in viral genome in determining response to different drug therapies.

Work done

During the current year, a total of 135 blood samples were received from the different collaborative centers. These included 91 samples from day 0, 35 samples from 12 weeks, 9 samples from 24 weeks. Out of these 114 samples were positive for HBV DNA. Eighty-two PCR positive samples were further processed for genotyping. Out of these samples, 50 belonged to genotype D, 23 belonged to genotype A and the rest 9 samples belonged to genotype C.

Services Provided

- **Testing for Drug Controller of India:** A total of 426 blood products submitted by the drug controller of India were tested for HBsAg and HCV RNA and reports submitted.
- **Chronic Hepatitis B and C patients:** A total of 52 and 53 patients were tested for the presence of HCV RNA and HBV DNA respectively in PCR. HBV quantitation was done for 56 samples.
- **Sporadic acute viral Hepatitis Patients:** A total of 375 and 272 & 96 serum samples were tested for the detection of anti-HAV-IgM, anti-HEV-IgM and HBsAg respectively.
- **Epidemics of viral Hepatitis:** Total 420 sera representing 7 outbreaks of viral hepatitis were tested which were either due to hepatic E or A viruses.
- **Core sequencing facility:** The core sequencing facility (3130XL Genetic Analyzer) present in the department was used to provide sequence analysis for 11,892 samples provided by other departments.

Publications

- Kulkarni MA, Arankalle VA. The detection and characterization of Hepatitis E virus in pig livers from retail markets of India. **J Med Virol**. 2008 Aug; 80(8): 1387-90.
- Arankalle VA, Lole KS, Deshmukh TM, Chobe LP, Gandhe SS. Evaluation of human (genotype 1) and swine (genotype 4)-ORF2-based ELISAs for anti-HEV IgM and IgG detection in an endemic country and search for type 4 human HEV infections. **J Viral Hepat**. 2007 Jun; 14(6): 435-45.
- Deshmukh TM, Lole KS, Tripathy AS, Arankalle VA. Immunogenicity of candidate Hepatitis E virus DNA vaccine expressing complete and truncated ORF2 in mice. **Vaccine.** 2007 May 30; 25(22): 4350-60.
- Arankalle VA, Shrivastava S, Cherian S, Gunjikar RS, Walimbe AM, Jadhav SM, et al. Genetic divergence of Chikungunya viruses in India (1963-2006) with special reference to the 2005-2006 explosive epidemic. **J Gen**

Virol. 2007 Jul; 88(Pt 7): 1967-76.

- Arankalle VA. Hepatitis Viruses: Virology and Epidemiology. **Textbook of Tropical Hepatogastroenterology**, Chapter 14:261-268, Editor Dr. BN. Tandon, Elsevier publications.
- Arankalle VA, Gandhe SS. Virological diagnosis of hepatitis. **Textbook of Tropical Hepatogastroenterology,** Chapter 15:269-276, Editor Dr. BN. Tandon, Elsevier publications.

Workshops / Conferences / Seminar / Meetings attended

Dr. VA Arankalle;

- Presented hepatitis vaccine development related work done at NIV during an Indo-German (ICMR-HGF) meeting at ICMR, New Delhi on 3rd April 2007.
- Task Force meeting for the Project "Epidemiology of Viral Hepatitis in Tribes of Orissa, Madhya Pradesh/ Chattisgarh and Jharkhand, India" at Institute of Post Graduate Medical Education and Research, Kolkata held on 1st May 2007
- Poster presentation (Title of the poster "Genetic analysis of Influenza-A viruses isolated during an outbreak of encephalitis in children from West Bengal, India, 2003".) during the conference on "Options for the Control of Influenza VI" Held at Toronto, Ontario, Canada during June 17-23, 2007
- Meeting with WHO representatives on "Vaccines" at ICMR, New Delhi on 22nd August 2007
- Presented surveillance for Hepatitis at meeting held at ICMR (ISDP), New Delhi on 7th September 2007
- Meeting of the project " A multicentric randomized controlled clinical trial of adefovir, adefovir +lamivudine, and combination of adefovir and glycyrrhizin in HBV related decompensated cirrhosis", at ICMR on 17th September 2007.
- Talk on Hepatitis C vaccine: Current status during Workshop on 'Vaccines & Anti-infectives' under ICMR HGF, in Germany during 30-31 October 2007.
- Invited speaker (Topic: Hepatitis A Epidemiology: India) at "A Global Hepatitis A meeting "at Miali, Florida, USA on November 30-December 1, 2007.

Dr. KS Lole

- Training programme on 'Leadership for Senior Women Scientist', at Administrative Staff College of India, Hyderabad during July 23-27, 2007.
- International Conference on 'Emerging and Re-Emerging Viral Diseases of the Tropics and Sub-Tropics', at New Delhi December 11-14, 2007.

Dr. AS Tripathy

- Training programme on 'Flow cytometry', BD Pharmingen, New Delhi during July 2-4, 2007
- Oral presentation entitled 'Cellular immune responses to ORF-2 and NE proteins in primates with hepatitis E infection and vaccination' at '34th Indian Immunology Society Conference', at National AIDS Research Institute, Pune, during December 16-18, 2007.

Dr. LP Chobe

- Presented poster titled 'Hepatitis A outbreak from Shimla'. Himachal Pradesh' at International Conference on 'Emerging and Re-Emerging Viral Diseases of the Tropics and Sub-Tropics', at New Delhi December 11-14, 2007.

Dr.TM Deshmukh

- Training programme on 'Patent Drafting', conducted by CSIR at NISCAIR, New Delhi, during October 22-26, 2007

Shubham Srivastava

- Training programme on 'Taqman Low Density Array', at Labindia, New Delhi during September 10- 14, 2007.
- Presented poster titled 'Enhancement of humoral immune response to a combination vaccine against Hepatitis B surface antigen (HBsAg) and Hepatitis E partial capsid protein NE', at New Delhi December 11-14, 2007.
- Oral presentation entitled 'Study of immune response to a combination vaccine against Hepatitis B and Hepatitis E in mice'. (Judged as the third best presentation in Ph D student category) at '34th Indian Immunology Society Conference', at National AIDS Research Institute, Pune, during December 16-18, 2007.

Subhashis Chatterjee

- Presented poster titled 'Detection of antibodies induced by vaccine and hepatitis E virus infection' at International Conference on 'Emerging and Re-Emerging Viral Diseases of the Tropics and Sub-Tropics', at New Delhi December 11-14, 2007.

Training programmes / Workshops/ Seminars organized

Workshop on 'Molecular Biology Techniques for Hepatitis Viruses' was conducted in hepatitis division for the project staff engaged at different centers in the multicentric task force project on 'Epidemiology of Viral hepatitis in Tribals of Orissa, Madhya Pradesh / Chattisgarh and Jharkhand, India', June 11-15, 2007

Japanese Encephalitis

Japanese Encephalitis

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Mr. Roopesh Gangwar
Ms. S. Harini

Project Fellows

Mr. Pradip Fulmali

3 Japanese Encephalitis

- Investigation of an outbreak of encephalitis in Gorakhpur, UP
- Molecular characterization of newly identified viruses
- Epidemiology and immune response against Japanese encephalitis virus strains at molecular level in North-Eastern regions of India
- Determinants of peripheral pathogenicity of West Nile virus
- Evaluation of the protective ability of immune cells against the challenge of WN virus strains Eg - 101 and 68856
- Immunoprotective mechanism in mouse model of JE virus
- Differential expression of genes in mouse brain infected with JE virus
- Post marketing study to evaluate the safety and immunogenicity of a single dose of JE vaccine at Burdwan, West Bengal
- A prospective open-label, uncontrolled, single-centre, evaluation of viraemia in healthy flavivirus seronegative adults after primary vaccination with JE SA14-14-2 live attenuated vaccine (JEATTE/IND/01- PUNE)
- Selective Expression of Recombinant Viral Protein in Immunocompetent Cells
- Development of molecular techniques for the rapid detection of agents infecting the central nervous system

Investigation of an outbreak of encephalitis in Gorakhpur, UP

MM Gore, VP Bondre, GN Sapkal, PV Fulmali, V Shankaraman, VM Ayachit and D. Gangale.

NIV team deputed at Gorakhpur during August - September 2007 has investigated acute encephalitis syndrome (AES) cases for viral etiology. According to State Government Health Services, 2098 cases were admitted from January December 2007 in the region and 398 deaths (CFR 18.97%) were reported. A total number of 1277 clinical specimens which includes CSF (606), acute sera (601), rectal swabs (32), throat swabs (32) and brain biopsies (05) were collected. All the clinical specimens were tested for detection of etiological agent by various tests.

Investigation of specimens for Japanese encephalitis virus infection.

A total of 663 sera were tested for presence of anti JEV IgM antibodies. 63/663 (9.5%) CSF's and 70/646 (10.8%) sera were tested positive for anti JEV IgM antibodies. Most of these were serum-CSF pairs. Presence of JE virus genome in CSF and serum was tested by JEV specific RT-PCR and real time RT-PCR assays. 46/488 (9.4%) CSF's were tested positive for JE virus genome. 3/488 CSF's tested positive for both the anti JEV IgM antibodies and JEV specific real time RT-PCR. Thus total positivity for JEV etiology was about 18%. In addition 141/ 602 (23.42%) sera were tested positive for anti JEV antibodies by CPE neutralization assay.

Isolation of JE virus was attempted by inoculating CSF's in infant mice. Out of 76 CSF samples inoculated 23 suspected mouse brains were tested for presence of JEV by RT-PCR. Two isolates of JE virus have been obtained from 2007 outbreak. Sequence analysis of partial C - PrM region indicates close sequence similarities with GP-78 isolated from 1978 Gorakhpur epidemic.

Testing of specimens for enterovirus diagnosis

Along with JEV diagnosis, specimens were also tested for enterovirus diagnosis by RT-PCR. 10 / 488 CSFs tested positive by enterovirus specific generic RT-PCR. Sequence analysis indicated close similarities with EV76 (4), Echo 11 (3), EV84 (1), Cox 16 (1) and Echo 30 (1). In addition, EV 76/89 was detected in 2 /11 CSF specimens obtained during the month of March 2007.

Isolation of the viral agent from patient's CSF

Enterovirus isolation was attempted by inoculating CSF specimens in different cell lines. A total of 319 CSF's were inoculated in Vero E6, Rhabdo myosarcoma (RD) and BHK cell lines. 30 cultures showed some changes in the cell morphology after 2-3 passages. Out of these, two passage - 2 cultures showed EV like particles by electron microscopic analysis and EV was detected in 3 cultures by RT-PCR (2- EV 76 & Echo 11). Additionally JEV was detected by RT-PCR in 4 cultures. A set of CSFs were also tested for diagnosis of encephalitis associated RNA viruses including JE, Entero, Alpha, Nipah, Hendra by RT-PCR and DNA viruses including HSV and VZV by specific PCR assays.

Molecular characterization of newly identified viruses

Background

Complete genome sequence of newly identified Enterovirus 76 isolated from human clinical specimen collected during 2006 Gorakhpur encephalitis outbreak was obtained analyzed. Similarly complete genome sequence of

newly identified Bagaza virus isolated from *Culex tritaenorhynchus* mosquito pool collected during encephalitis outbreak in Kerala during 1996 was obtained and analyzed. Additionally complete genome sequence of highly pathogenic West Nile virus strain 68856 isolated from *Rousettus leschenaultia* (fruit bat) from Karnataka during 1968 was obtained and analyzed.

Molecular characterization of Enterovirus 76 isolated from 2006 Gorakhpur encephalitis outbreak

VP Bondre, GN Sapkal, PV Fulmali, V Shankaraman, VM Ayachit and MM Gore.

Background

Partial genome sequences obtained by EV specific RT-PCR from human clinical specimens and typing of the virus by partial VP1 sequence using tissues culture amplified viruses showed maximum similarities with human Enterovirus 76 first time documented in Bangladesh from acute flaccid paralysis cases during 1999-20001. Complete genome sequence of one of the Indian isolate was obtained and analyzed.

Objective

- Full length genome sequencing of Enterovirus 76 and data analysis.

Work done

Two tissue culture grown viruses (specimens 0623407 & 0623350) were processed for the genotyping among Enteroviruses by RT-PCR amplification of 890 nt product from VP1 / 2A region (2439-3329). The sequence analysis confirmed both the isolates as Human Enterovirus -76. Phylogenetic and nucleotide identity analysis indicate closer relationship with Human Enterovirus 76 isolated in Bangladesh during 1999-2000 **(Fig 1)**.

Primers for complete genomic sequencing of EV-76 isolate were designed by aligning all the available full genome sequences from GenBank. A 7400 nt sequence of isolate 0623407 was obtained from both the ends and analyzed. The sequence was compared with worldwide isolated Enteroviruses and its phylogenetic relationship was determined.

Findings

Complete genome sequence analysis of human EV isolated from GKP 2006 encephalitis outbreak revealed differences from most of the worldwide isolated Enterovirus strains. However, the Indian EV strain showed maximum sequence similarity of 91-92% with Enterovirus 76 isolated from Bangladesh during 1999 -2001 from acute flaccid paralysis cases.

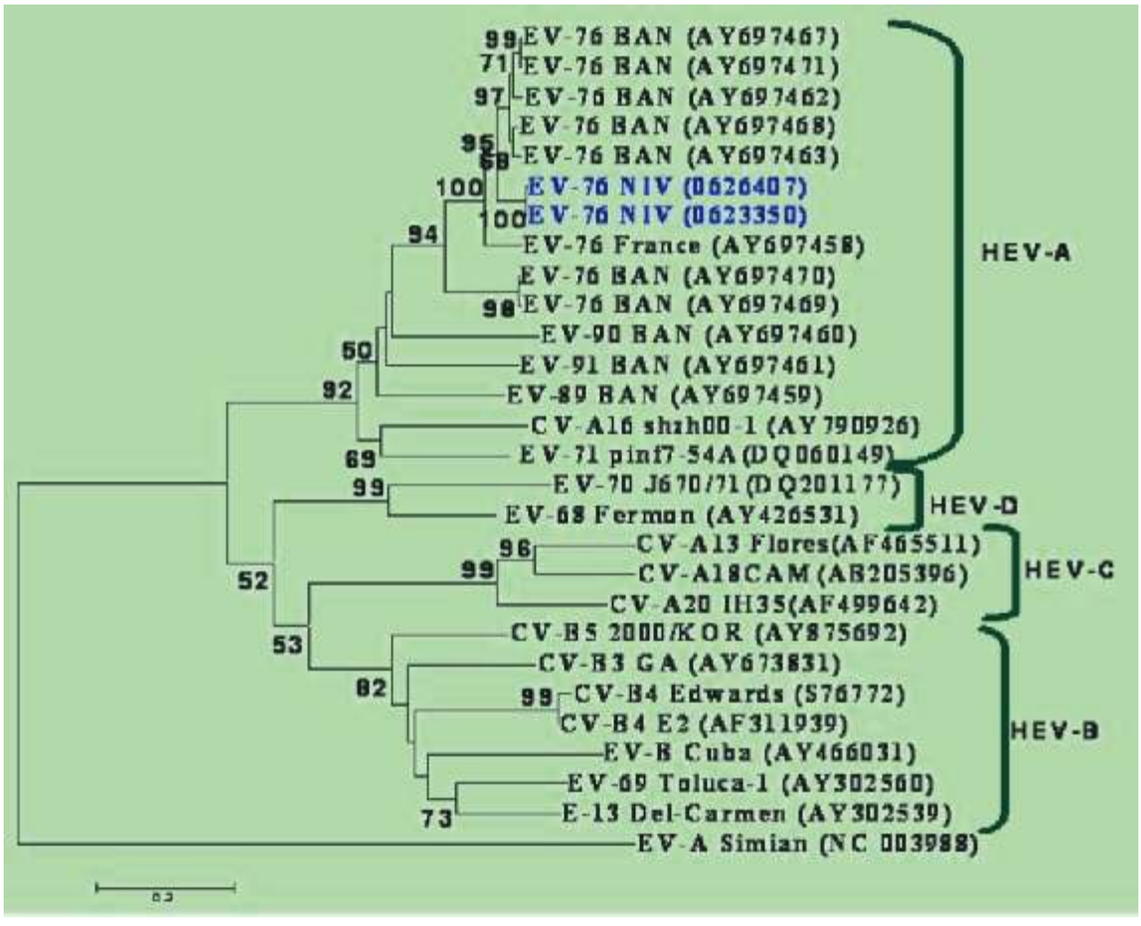


Fig. 1. Phylogenetic analysis of human enterovirus using 930 nt VP1/2A gene sequence.

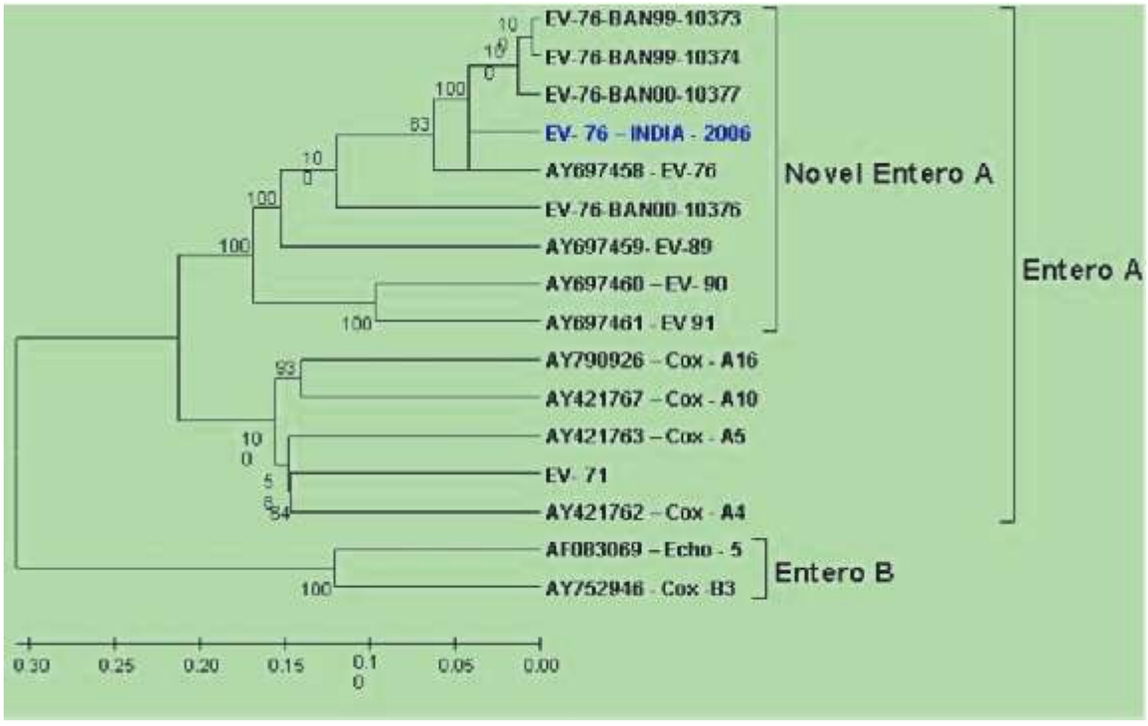


Fig.2. Phylogenetic analysis of human enterovirus 76 using complete genome sequence.

Genomic characterization of Bagaza (BAG) virus isolated from *Culex tritaenorhynchus* mosquitoes collected from Kerala during encephalitis outbreak

VP Bondre, GN Sapkal, Y. Prasanna, PV Fulmali, V. Shankaraman, D. Gangale, VM Ayachit, G. Jacob and MM Gore.

Background

An arbovirus was isolated from a pool of female *Culex tritaenorhynchus* mosquitoes collected from Punna Kunnam region of Allappuzha district of Kerala during an encephalitis outbreak in 1996. Preliminary serological tests including complement fixation and neutralization tests using hyper immune serum against JEV, WNV, CHPV, CHIKV, Sindbis, Bital and DENV 1, 2, 3, 4 characterized it as JEV and WNV cross reactive virus. Studies were carried out to determine the genetic and biological relationship of the arbovirus isolate with JE, WN and other flaviviruses.

Objective

- Molecular characterization of an arbovirus isolated from mosquitoes collected during an encephalitis outbreak in Kerala in 1996.

Work done

The evidence on the arbovirus isolate as Bagaza virus came from RT-PCR amplification and sequence analysis of NS5 region amplified using Flavivirus specific universal primers. 95% sequence similarities with Bagaza virus followed by 93% similarities with Israel turkey meningoencephalitis virus (ITV) suggests genetic relatedness of the isolate with Ntaya group of flaviviridae. Complete genome sequence of the newly characterized Bagaza virus was obtained by RT-PCR amplification and sequencing of genome using primers designed by aligning all the full length genome sequence of related viruses. The 10281 nt full genome sequence of Indian Bagaza virus (GenBank accession number EU684972) represents complete open reading frame (ORF) coding 3426 amino acids. Complete genome sequence analysis showed close similarities with Ntaya group of viruses with maximum similarity of 94.9% with African Bagaza virus - Kuno strain. However as compared to Kuno strain, Indian Bagaza virus showed 515 nucleotide changes leading to a total number of 77 amino acid alterations throughout the ORF. Additionally BAGV-Ind showed one deletion (7424) and 4 additions (7438-39, 7444 and 7463) at the C terminal region of NS4B encoding protein.

To investigate the phylogenetic relationship of Indian Bagaza virus with other members of flaviviridae, multiple nucleotide and putative amino acid alignments were made. Phylogenetic analysis based on complete ORF as well as partial nucleotide sequences and putative amino acid sequences generated close relationship of viruses transmitted by *Culex* mosquitoes, *Aedes* mosquitoes, ticks and unknown vectors. Both the African and Indian Bagaza virus isolates clustered together on phylogram constructed with complete ORF encoding sequence of various members of flaviviridae **(Fig. 3)**. Bagaza virus strains clustered together with members of *Culex* clade mostly causing encephalitis disease in man. Complete ORF sequence analysis of both Indian and African strains showed percent nucleotide identity (PNI) of 95±0.20% with each other. Partial envelope and NS5 sequence based analysis also showed similar phylogenetic relationship of BAGV with other memenrs of flaviviridae as obtained with complete ORF sequence analysis. Other members of Ntaya group including Tembusu virus, Ntaya virus and ITV also clustered with BAGV in phylograms constructed using partial envelope and NS5 sequences. Envelope

sequence comparison of BAGV-Ind with BAGV-Kuno (AF372407) and Dak- Ar-B209 (AF372407) strains showed PNI of 95.12±0.76 and 95.54±0.79% respectively while BAGV-Kuno showed PNI of 99.67±0.25% with Dak- Ar-B209. With partial NS5 sequence, BAGV-Ind showed PNI of 99.90±0.10% with BAGV-Kuno (AY632545) and Dak-Ar-B209 (AF013363) while 99.90±0.10% PNI was observed between BAGV-Kuno and Dak- Ar-B209.

Findings

The partial as well as complete genome sequence analysis of the arbovirus classified as Bagaza virus which is a member of Ntaya group of flaviviridae transmitted by *Culex* mosquitoes and are mostly characterized as encephalitic viruses.

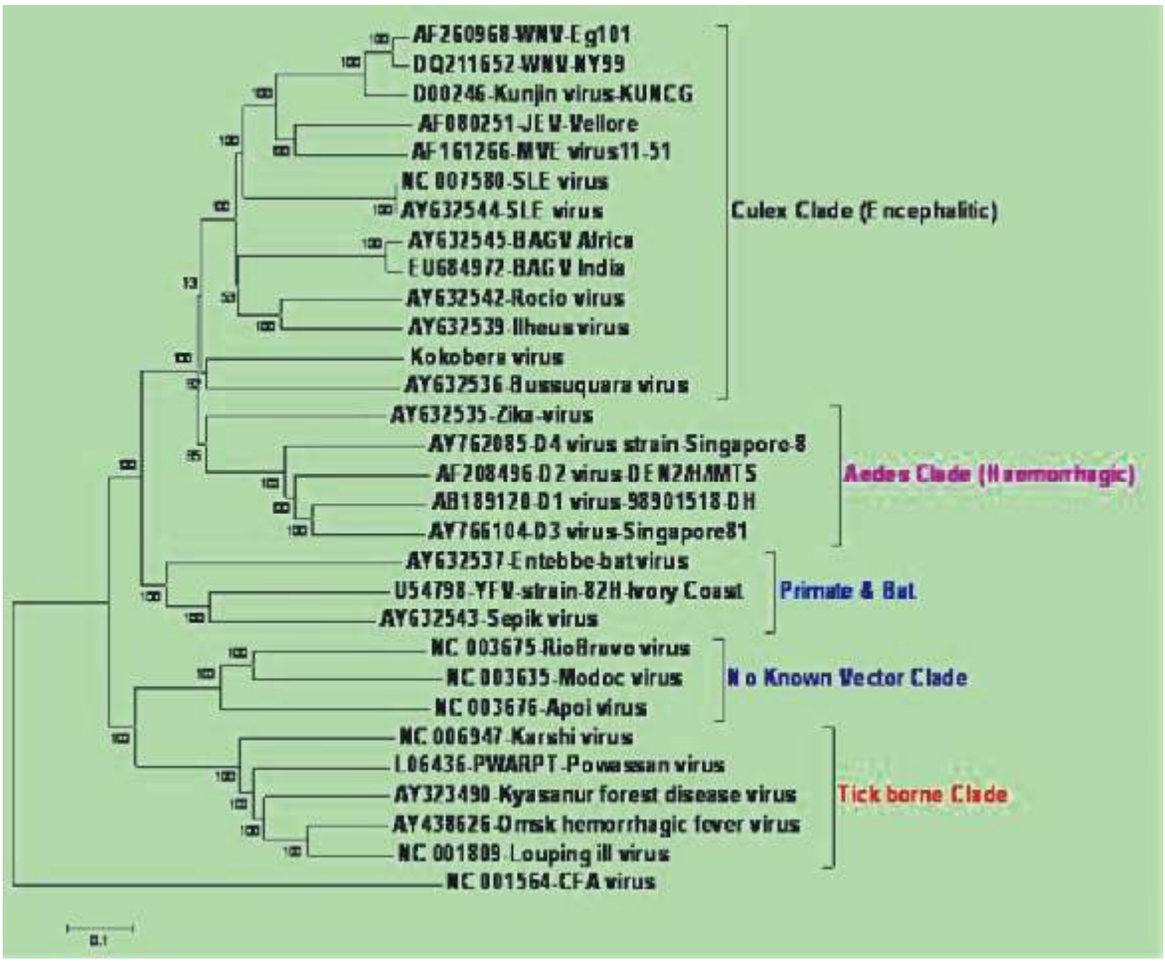


Fig. 3. Phylogenetic analysis of Bagaza virus using complete genome sequence.

Full genomic characterization of a highly virulent, lineage 1 West Nile virus (68856) strain isolated from bat

VP Bondre, GN Sapkal, V. Shankaraman, PV Fulmali, D. Gangale, VM Ayachit, and MM Gore.

Background

West Nile virus strain 68856 isolated from bat in 1968 is neurovirulent and peripherally pathogenic in mice. Several studies using 68856 characterized it as highly virulent strain as compared to other WN strains isolated in India. Comparative biologically characteristics are shown to be distinct from Egyptian prototype strain Eg-101. We have documented difference in cross neutralization assay of 68856 and Eg-101. Our genetic studies based on partial genomic sequence in C-prM-E region indicate that the strain is genetically distinct from other Indian strains and sequence similarity with Eg-101 was observed. However, genetic differences with Eg-101 are reported in NS1 region by researchers in other laboratories.

Work done

In addition to the difference in cross neutralization studies, difference in cross protection through immune lymphocyte was studied using immune cell transfer studies. In order to understand the sequence changes associated with altered biological properties of Eg-101 and 68856, we obtained complete genomic sequence of 68856. The sequence was compared with all sequences of globally reported human pathogenic and moderately pathogenic strains available in GenBank. The sequence showed 96% similarity with most of the lineage 1 epidemic strains isolated from humans in Western hemisphere. However complete genomic sequence of 68856 showed closer relationship with Eg-101 (Egypt) with 99% and Chin -01 strains with 97% similarities. As compared to Eg-101, altogether difference in eight nucleotides 2 in 5' NTR, 4 in Env, 1 in NS2A & 1 in NS5 coding regions was recorded that lead to difference in 5 amino acids 4 in Env and 1 in NS2A protein coding regions. As compared to Eg-101 complete amino acid coding ORF, the 68856 showed 4 amino acid changes in envelope region at P156-S, T332-K, T366-A, H398-Y and one change at V138-A in NS2A region. Among Flaviviruses, amino acids 154-156 fall in NYS motif involved in receptor and antibody binding. The altered residues in the receptor-binding domain determine virulence of the strains. Alteration of S156 to F or P or any other amino acid residue results in neurovirulence property of the strain. Studies replacing S156 to other amino acids by site directed mutagenesis altered the virulence in animal experimentations. Role of other amino acid changes T-K, T-A, H-Y and V-A in determining the biological properties of WN virus are not yet clear.

Findings

Complete genome sequence analysis of West Nile virus strain 68856 shows 26% genetic divergence with Indian WNV isolates and close similarity with lineage 1 pathogenic strains. Detailed studies are necessary to determine the role of P-S alteration in virulence of the virus.

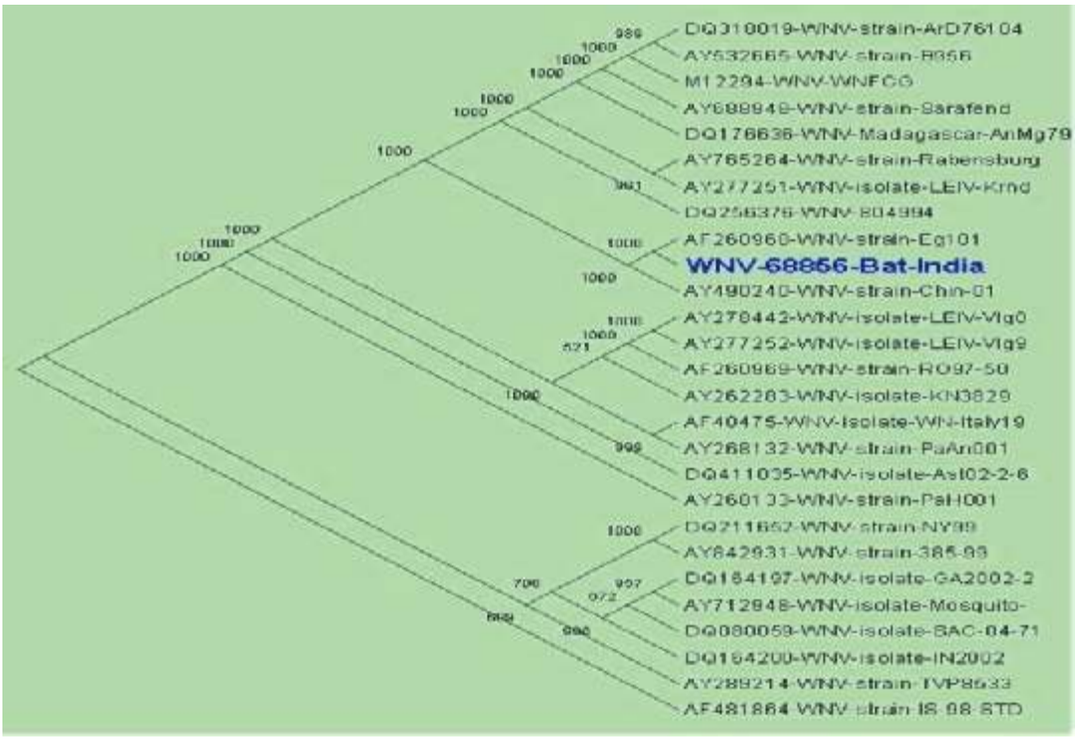


Fig.4. Phylogenetic analysis of WNV 68856 strain using full genome sequence.

Epidemiology and immune response against Japanese encephalitis virus strains at molecular level in North-Eastern regions of India

MM Gore, VP Bondre, SA Khan, P. Dutta, GN Sapkal, PV Fulmali, D. Gangale, V. Shakaraman, VM Ayachit and RS Gangwar

Virus isolation and detection of JE and WN virus from patient's CSF

332 Cerebrospinal fluid and 200 sera were collected from suspected encephalitis patient during May-Aug 2007 in northeastern region. The CSF's were processed for virus isolation by inoculation in infant mice and susceptible cell lines. Brains harvested from suspected sick mice were tested for the detection of JE and WN viruses by virus specific single step RT-PCR. Five isolates characterized as 3 JE virus and 2 WN virus have been established in infant mouse brain. The JE and WN virus isolates are being genetically characterized by sequence analysis. Limited sequences analysis indicate closer relationship of JE virus isolates with Indian prototype strain P20778 and that of WN virus isolates with Indian isolate 804994. Further studies are in progress.

Diagnostic RT PCR of CSF's collected in the acute phase of encephalitis for JE and WN virus

Viral RNA isolated from all the 332 CSF's was processed for diagnosis of JE and WN virus infections by JE and WN virus specific RT-PCR followed by semi-nested PCR. 46/332 (13.85%) CSF's were positive for JE virus specific PCR and 9/332 (2.7%) CSF's were positive for WN virus specific PCR. All the PCR products amplified directly from CSF were sequenced and analyzed. Phylogenetic analysis based on 250 nt product amplified by semi-nested PCR directly from CSF indicate genetic similarities with most of the circulating Indian strains **(Fig. 5)**. However, three JEV sequences obtained directly from CSF were genetically distinct from strains circulating in India. Since this data is based on small genomic fragment which has to be supplemented with additional data by amplifying other larger genomic regions of the virus directly from CSF. Further studies on isolation and genetic characterization are in progress.

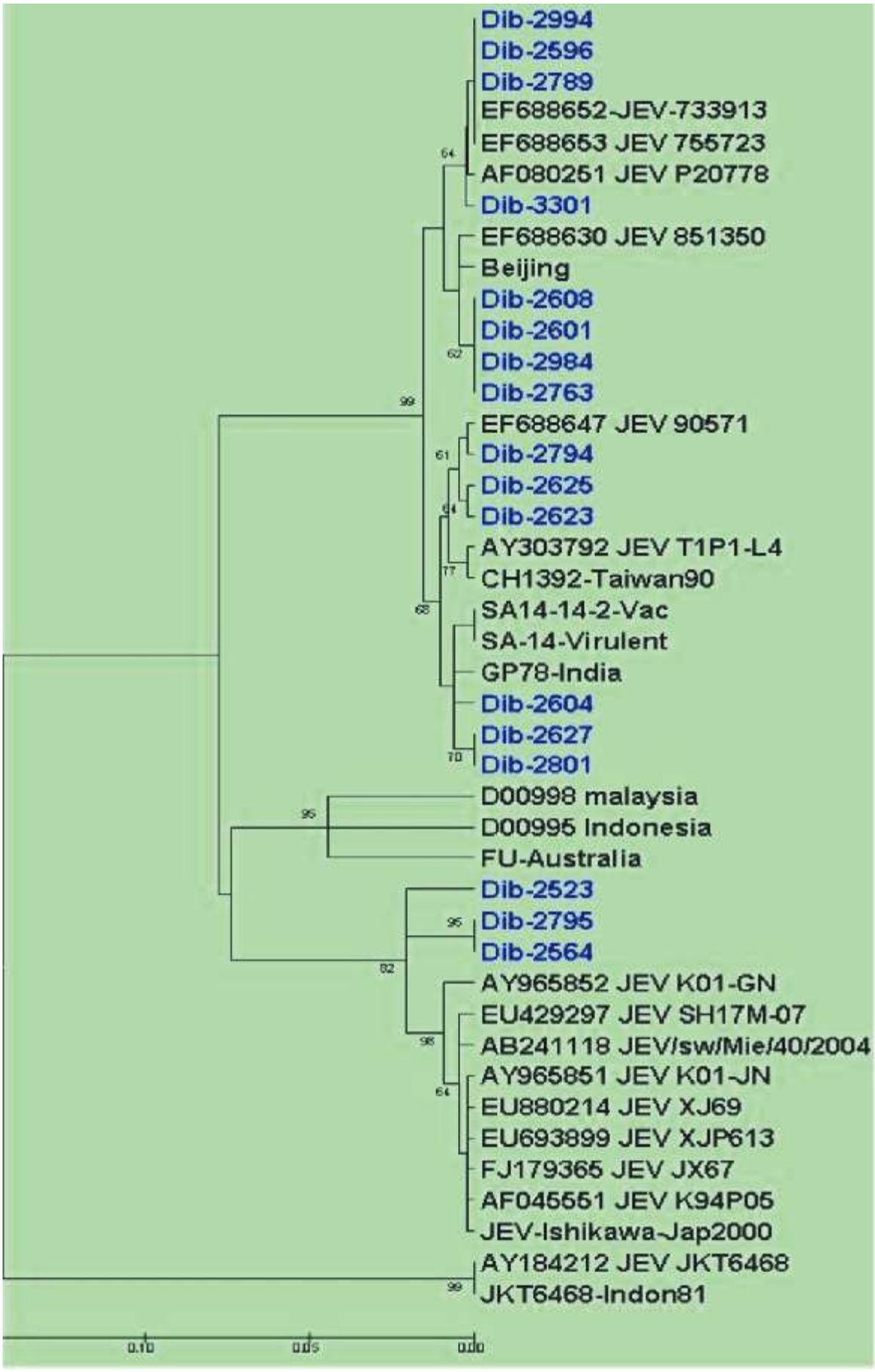


Fig.5. Phylogenetic analysis of JE sequences directly amplified from patients

Detection of anti WN virus specific IgM antibodies

The 332 CSF specimens and 200 sera were tested for detection of anti JE virus specific IgM antibodies using NIV kit at RMRC Dibrugadh. 245 / 434 CSF (56%) positive and 191 / 593 sera (32.2%) were positive for JE IgM antibodies. Additionally sera were tested for WN virus specific IgM antibodies detection using West Nile virus specific PAN-BIO Kit. 6/123 (4.87%) sera tested positive for anti WN virus specific IgM antibodies. Further serological investigations of CSF and sera are in progress.

Prevalence of JEV and WNV specific neutralizing antibodies among children in Dibrugarh.

Sera collected from children from Dibrugarh area during the year 2006 were analyzed for estimation of neutralizing antibodies against JE (733913) and WN (68856) by CPE inhibition assay. 55.87% of children (1002 sera tested) showed neutralizing antibodies against JE while 43.5% sera showed WNV specific neutralizing antibodies. 36.42% of the children did not show neutralizing antibodies against these viruses. Prevalence of JEV and WNV specific neutralizing antibodies was documented in 20.05% and 7.68% of the sera respectively. The data warns necessity of further studies to understand circulation of these viruses, their distribution and genetic relationship with other strains documented in country.

Table 1 . JEV and WNV neutralizing antibody titers among children sera from Dibrugarh.

<table>
<tr><th colspan="2" rowspan="2">Category</th><th rowspan="2">Ab Titer</th><th colspan="2" rowspan="2">Number of Samples</th><th colspan="2">% positivity within the</th></tr>
<tr><th>Sample gr</th><th>Total gr</th></tr>
<tr><td rowspan="2">JE alone</td><td>High Titer</td><td>>40</td><td rowspan="2">201</td><td>122</td><td rowspan="2">20.05%</td><td>60.69%</td></tr>
<tr><td>Low Titer</td><td>20-40</td><td>79</td><td>39%</td></tr>
<tr><td rowspan="2">WN alone</td><td>High Titer</td><td>>40</td><td rowspan="2">77</td><td>58</td><td rowspan="2">7.68 %</td><td>75%</td></tr>
<tr><td>Low Titer</td><td>20-40</td><td>19</td><td>25%</td></tr>
<tr><td rowspan="4">JE and WN both</td><td>High Titer</td><td>>40</td><td rowspan="4">359</td><td>41</td><td rowspan="4">35.82%</td><td>11%</td></tr>
<tr><td>Low Titer</td><td>20-40</td><td>160</td><td>45%</td></tr>
<tr><td>High for JE & low for WN</td><td>>40 (JE) 20-40(WN)</td><td>136</td><td>38%</td></tr>
<tr><td>High for WN & low for JE</td><td>>40 (WN) 20-40(JE)</td><td>22</td><td>6%</td></tr>
<tr><td colspan="2">Total Negative</td><td><20</td><td colspan="2">365</td><td colspan="2">36.42%</td></tr>
<tr><td colspan="2">Total</td><td></td><td colspan="2">1002</td><td colspan="2"></td></tr>
</table>

Virus isolation attempts from field collected mosquitoes

Mosquitoes collected during the outbreak season were identified and 127 mosquito pools were made. 7 pools were tested positive for JEV by antigen capture ELISA. These pools were also processed for virus isolation in infant mice and RT-PCR diagnosis. No isolation was obtained up to passage 2. Similarly these mosquito pools and infected mice brains were negative by JEV specific RT-PCR.

Standardization of ELISA using synthetic peptide (B cell)

After standardization of ELISA using patient's sera with the B cell peptides from JE, WN and DEN viruses, the test was further standardized using available survey sera with known neutralizing antibody titers. The following peptides were selected for further study after primary screening, which were used for standardization of peptide ELISA. Out of 42 peptides, a set of eight peptides that showed type specific reactions were selected for ELISA against JEV using pooled sera.

JE Specific Peptides

JE Egp 40-TLDVRMINIEASQLA-54	1JE40
JE Egp 149-SENHGNYSAQVGASQA-164	3JE149
JE Egp 288-RLKMDKLALKG-298	6JE288
JE Pre M Aligned 33-PTSKGENRG-41	42JP33

WN Specific peptides

WN Egp 147-TTVESHGKIGATQAGRF-163	11WE147
WN Egp 302-SKAFKFARTPADTG-315	14WE302
WN Pre M Aligned 19-VTDVITIPTA-28	39WP19
WN Pre M Aligned 93-SLTVQTHGESTLA-105	41WP93

The survey sera (Warangal region) with predetermined neutralizing antibody titers against JE (733913) and WN (G22886) were used on these peptides for ELISA. The antigen capture ELISA was carried out with JE (733913) and WN (G22886) viruses (captured on Hx2 cross reactive monoclonal antibody) to determine the appropriate dilution and cross reactivity of these sera in the peptide ELISA.

The NT titers of the sera were as follows :

Serum samples	JE NT titer	WN NT titer
JE (733913) Positive	>1250	30-150
WN (G22886) Positive	<10	>1250

The sera having neutralizing antibodies against WNV did not cross react with JEV peptides. However, JEV positive sera showed cross - reactivity with WNV in neutralization. Hence, these sera were selected for further standardization of peptide ELISA.

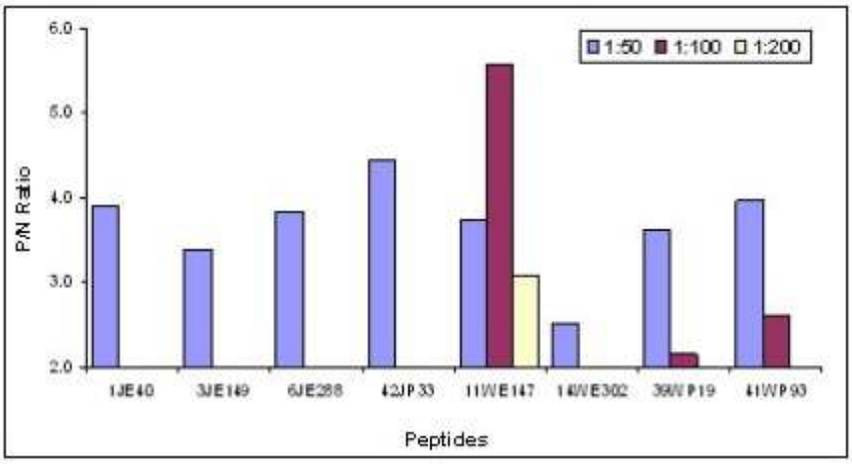


Fig. 6. Reactivity of human sera with JE and WN specific peptides in ELISA.

The peptide ELISA showed similar results with the whole virus. WNV specific peptides 11WE147 showed specific reactivity with WN Nab positive sera.

All the reagents including blocking agents (1-1.5% Gelatin, 1-2% BSA, 1-2% skimmed milk), washing solutions (0.05-0.1% T-20 with and without salt) and reaction conditions (temperature) were standardized to minimize the background noise in peptide ELISA. 1% skimmed milk as blocking agent and 0.1% T-20 as wash buffer gave optimum results in peptide ELISA whereas different salt concentrations in washing buffer did not have any effect on ELISA.

Cross-reactive epitope on JE Egp and Histone

During generation of monoclonal antibodies (MAbs) against envelope protein of JEV, two MAbs (NHA-1 and NHA-2) were obtained that were cross reactive with cellular histones. These findings were confirmed by studying the Ab3 response against anti idiotypic antibody against MAb NHA-1. Preliminary analysis suggests that the reactive epitope on JEV envelope protein might be non-sequential which is reacting with histone in western blotting. This may be due to a partial conformational and structural homology between reactive epitopes of histone and JEV envelope protein. JEV envelope protein has been demonstrated in the nucleus of infected cells, which may due to transportation in nucleus because of their structural homology. It would be interesting to understand whether the non-sequential epitopes on JEV is mimicking the sequential epitope on histone proteins. This study will lead to understand the reason of translocation JEV envelope protein to nucleus.

Determination of homology between histone and JEV envelope protein.

Amino acid alignment analysis of JEV envelope protein and hostone protein does not show simple sequence-level homology. Similarly these proteins did not have any homology between conserved regions. Similar kind of cross - reactivity between histone like protein (HLP) from *Mycobacterium tuberculosis* with these MAbs (NHA-1 and NHA-2) have been documented. A sequence similarity search using tetrapeptides from the envelope protein (after normalizing the amino acids according to their properties) gave the regions on the HLP that might be antigenic region on the histone. These have been selected for the study. The tetrapeptides sliding homology of JEV envelope protein with HLP after coding the sequences according to the above table has given the following results. On the bases of this homology following regions have been selected from HLP for this study:

HP_1: 06- LIDVLTQKLG -15

HP_2: 27- NVVDTIVRAVHKGDS -41

HP_3: 97- AEGPAVKRGVGAS -109

HP_4: 165- AKKVTKAVKKTAVKASVTKA -184

ELISA with histone peptides:

ELISA on histone peptides were carried out in nunc covalink ELISA modules. The coated peptides (Histone and JEV were also coated) were probed with NHA-1, NHA-2, HX-2 and JE polyclonal sera.

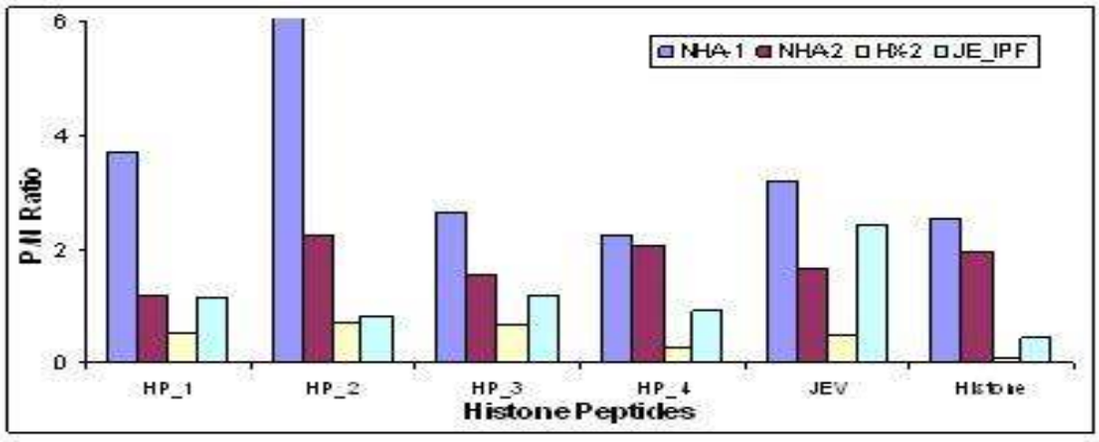


Fig. 7. ELISA on histone Peptides.

Findings

Histone peptides, histone and JEV (733913) strongly react with NHA-1 and NHA-2 monoclonal Abs. A low level of reactivity with anti - JEV polyclonal sera was observed. HP_1 and HP_2 showed higher reactivity than other peptides. No significant reactivity of HX-2 MAb was observed with any of the histone peptide or histone protein. These finding suggests that the envelope protein of JEV and histone protein might be sharing homology at structural level.

Determinants of peripheral pathogenicity of West Nile virus

GN Sapkal, VM Ayachit, VP Bondre and MM Gore.

Background

In order to determine the amino acids responsible for attenuation of West Nile virus, complete envelope proteins (E protein) coding sequence of the wild type strains WN 68856 and its neutralization escape mutants were analyzed. All these mutants showed common amino acid substitutions at position 89 Alanine- Phenylalanine, 90 Phenylalanine -Leucine, 91 Valine- Leucine. However, mutant IF1A71.1 showed two amino acid substitutions at positions 156 Serine-Proline, 242 Phenylalanine-Tryptophane which were absent in other two mutants. Of note is S156P substitution has been reported earlier in Flaviviruses and found to be involved in loss of peripheral virulence.

Objective

- To determine the altered cell tropism of mutant viruses with changes in E protein.

Work done

In our earlier work monoclonal antibodies exhibited less protection against the corresponding variants with altered pathogenicity as compared to the parent strain. Therefore, the present work was aimed to study an *In vitro* model to study the WNV infection in various cells. Two Nt escape mutants viz. NtE IF1A7 and NtE IVC3F10 along with the wild type WN 68856 viruses were used for study.

Peripheral infection of WNV encounters peritoneal exudates cells (PEC) and spleenocytes as a primary multiplication site that determines the outcome. Thus, growth of these mutants in PEC,adherent and non-adherent population of splenocytes was studied.

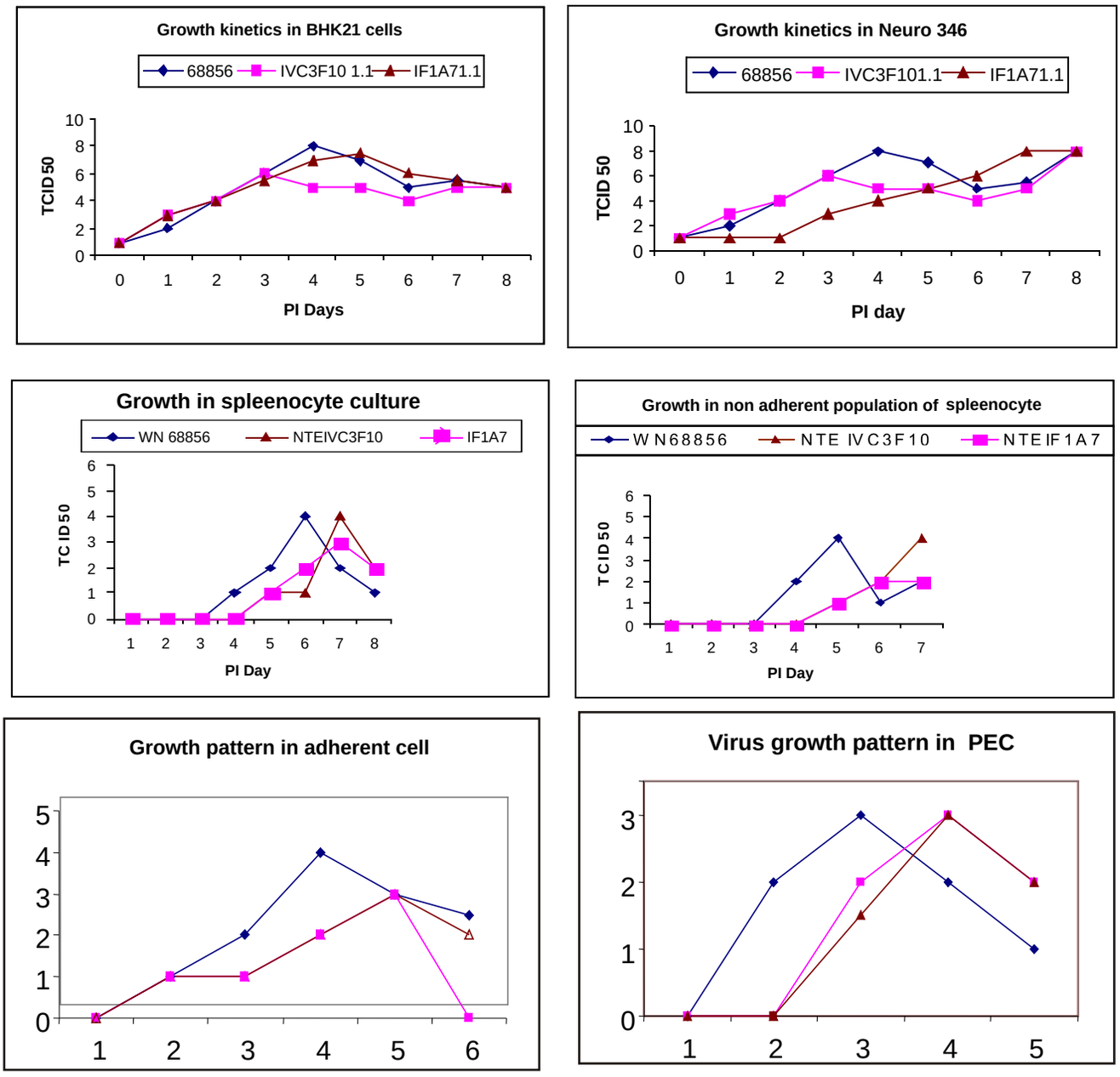


Fig. 8. Altered cell tropism of mutant viruses.

It was observed that the peripherally pathogenic WN 68856 shows significant titers within 24 hrs and 48 hrs and attains its maximum peak by 72 hrs in both splenocytes and PEC while the two mutant strains exhibit a very low titer in 24, 48 & 72 hrs and attain their maximum peaks only by 96hrs **(Fig.8)**. Thus the peripherally less pathogenic WNV exhibited a delayed release from the infected cells.

Although both the mutants retained high level of infectivity in Baby Hamster Kidney (BHK 21) cells, IF1A71.1 mutant exhibit delayed release in mouse neuroblastoma (Neuro 346) cells as compared to wild type and IVC3F101.2 mutant viruses. It appears that efficiency of replication in Neuron346 is likely to be a primary determinant of virulence.

When we compared growth pattern in splenocyte culture and its adherent, non-adherent populations, parent and mutant viruses exhibited different kinetics of replication **(Fig. 8)**. It appears that *In vitro* a wide variety of cell types successfully supports the virus replication, possibly through use of a variety of receptors of variable efficiency. It is possible that by releasing the higher amount of virus at the primary multiplication site in early phase of the infection

might be responsible for entry of the virus in brain. In addition, higher growth rate of wild type virus in neuronal cells would also contribute to the peripheral pathogenicity of the virus. Thus limiting the growth at the primary multiplication site (PEC) might be responsible for loss of pathogenicity.

Findings

Changes in amino acid domains of the E- protein might change the native conformation of the protein resulting in altered binding affinity of E- protein with its receptor. The delay in replication for mutant under conditions of high as well as low MOI suggests that the efficiency of penetration may differ as well.

Evaluation of the protective ability of immune cells against the challenge of WN virus strains Eg - 101 and 68856

SM Biswas, GN Sapkal, P. Lalwani, VM Ayachit, VP Bondre and MM Gore.

Background

Differences in genetic and antigenic nature of Egyptian prototype strain Eg-101 and Indian pathogenic strain 68856 prompted us to evaluate the role of immune splenocytes in protection against WNV by adoptive transfer.

Objective

- Determining role of the immune splenocytes in protection against WNV infection in mice.

Work done

Spleens from mice (BALB/c) immunized with three doses of Eg-101 (1000 LD_{50}) strain were harvested on 7th day post final immunization. Approximately 5x10^7 immune cells were adoptively transferred to irradiated mice via i.v. route which were challenged after 24 hrs with 50 LD_{50} of either Eg-101 or 68856 strains. Mice were observed up to 28 days for survival of challenge.

Findings

Challenge by homologous strain (Eg-101) induced 60% protection while in heterologous challenge by 68856 strain showed only 37.5% protection. Similarly challenge by JEV strain 733913 by i.p + ic routes showed only 25% protection.

Transfer of CD4 depleted and CD8 depleted cell population of WNV E-101 immune mice

Lymphocytes subpopulations isolated from immune splenocytes were separated by magnetic cell separation method and used for cell transfer and challenge studies**.** 75% of mice, which had been transferred with CD4+ depleted splenocytes, were protected from homologous WN E-101 challenge. CD8+ depleted splenocytes were, however not effective in mediating protection against homologous challenge WN E-101 **(Fig. 9)**

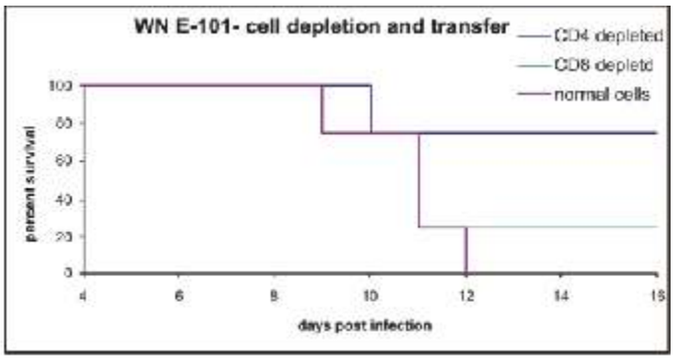


Fig. 9. Survival curve of mice lethally challenged with WNV Eg101 after adoptive transfer of CD4 and CD8 depleted splenocytes.

Immunoprotective mechanism in mouse model of JE virus

M Biswas and MM Gore.

Determining the role of CMI in protection against JEV infection in mice.

Mechanism of protection from lethal challenge of JE virus is less understood. Using cell transfer methods role of CMI can be studied.The study would be useful in understanding JE pathogenesis and immunodominant epitopes.

Objectives

To understand the role of CMI in protection of JE in mice.

Work done

Determination of virus titers in organs of mice receiving immune and non-immune splenocytes:

Viral replication in the peripheral organs and in the CNS of mice receiving immune or non-immune splenocytes was studied. Viral loads were detected by real time PCR using JEV specific primers and probe.

Fig. 10a

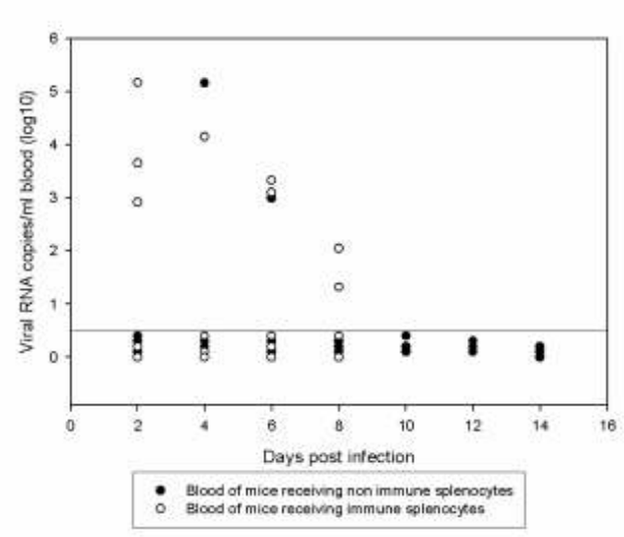


Fig. 10b

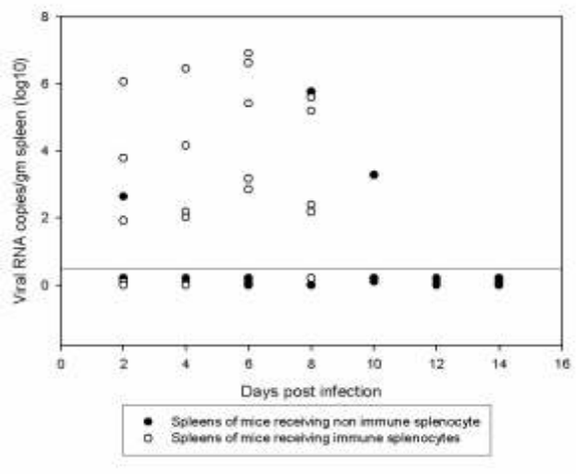


Fig. 10c

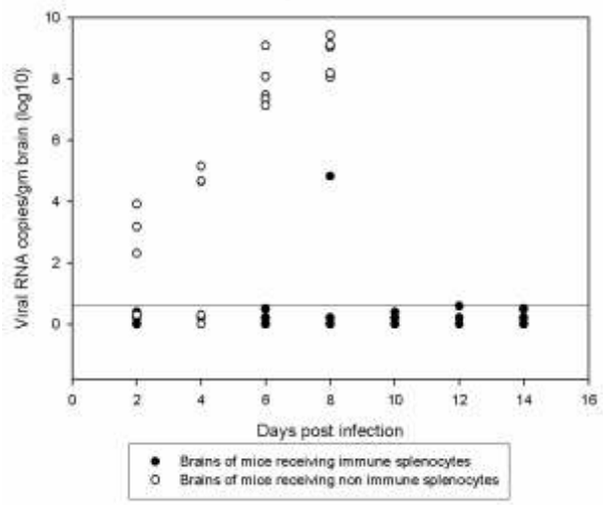


Fig.10. Scatter plots showing viral RNA copies in the a) Blood, b) Spleen, and c) Brain of mice receiving either JEV non-immune or immune splenocytes, taken at various time points PI. Each circle represents pooled organs from 2 mice.

Increased production of pro-inflammatory cytokines in infected mice:

Th1 and Th2 cytokines produced in response to JEV infection were determined by the CBA kit in the sera of mice receiving either immune or non-immune splenocytes. Higher levels of pro-inflammatory cytokines TNF-α ($p< 0.05$) and IFN-γ were observed in the sera of infected mice as disease progressed. TNF-α and IFN- γ expression levels seemed to parallel the progression of disease in non-immune mice, with baseline levels on day 2 PI continuously increasing, concurrent with the death of the animal. Levels of IL-2, IL-4 and IL-5 were much lower and peaked earlier during the course of infection (4th day PI), reaching basal levels by days 6 and 8 PI. Late in the course of infection, expression of IL-4 and IL-5 was seen to decrease in the sera of mice receiving non-immune splenocytes

Fig. 11a.

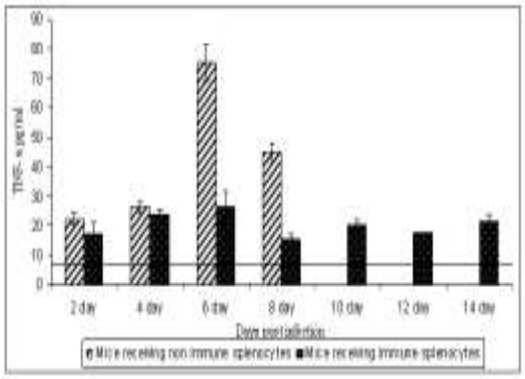


Fig. 11b.

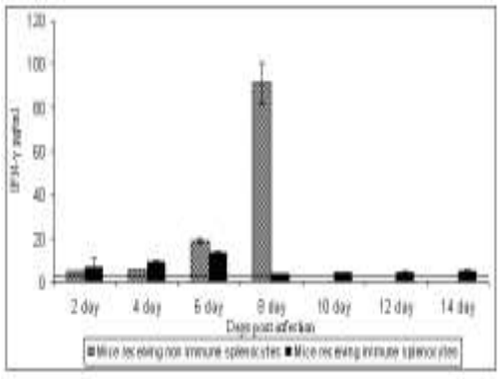


Fig. 11c.

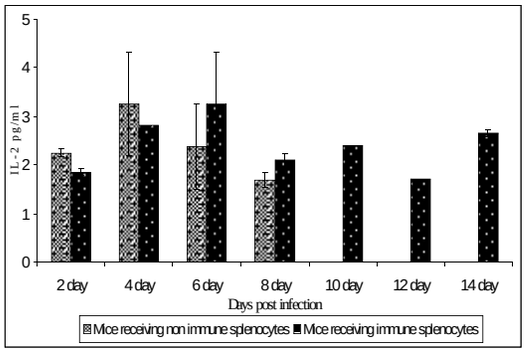


Fig. 11d.

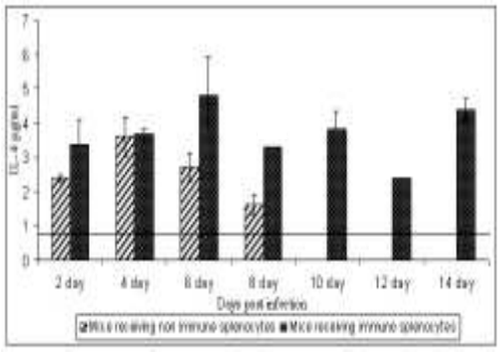


Fig. 11e.

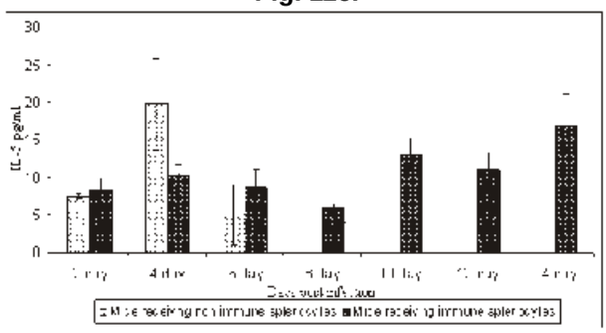


Fig. 11. Induction of pro and anti-inflammatory cytokines a) TNF- ? b) IFN- ?c) IL-2, d) IL-4, and e) IL-5 in the sera of mice receiving either non-immune or immune splenocytes.

Increased production of IgG1 antibodies in mice receiving immune splenocytes.

Induction of the antibody response after JEV infection in mice receiving either non-immune or immune cells was determined. In non-immune mice, IgM antibody reached titres of 1/400 by day 8 PI **(Fig. 12).** In mice receiving immune splenocytes before challenge with JEV **(Fig. 13)**, an elevated level of IgM antibody was observed with titres that were higher overall (1/560) than those observed in mice receiving non-immune splenocytes.

Antibody isotyping of IgG subtypes in mice receiving either non-immune or immune splenocytes revealed a significant increase in levels of IgG1 in the group that received immune splenocytes **(Fig. 13).**

IgG1/IgG2a ratios in mice receiving either non-immune or immune cells were calculated **(Fig. 14).** In contrast to non-immune mice, which showed a constant IgG1/IgG2a ratio at all time points PI, mice receiving immune splenocytes showed an increased ratio indicating increased IgG1 titres that suggested a switch towards a Th2 response.

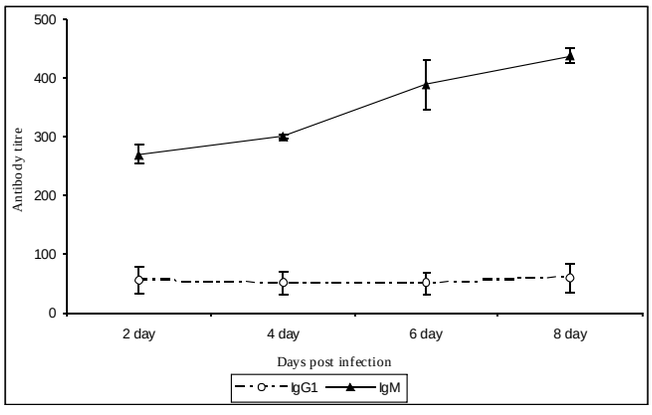


Fig. 12. IgM and IgG1 titres from pooled sera of JEV infected mice, collected at different time points post infection.

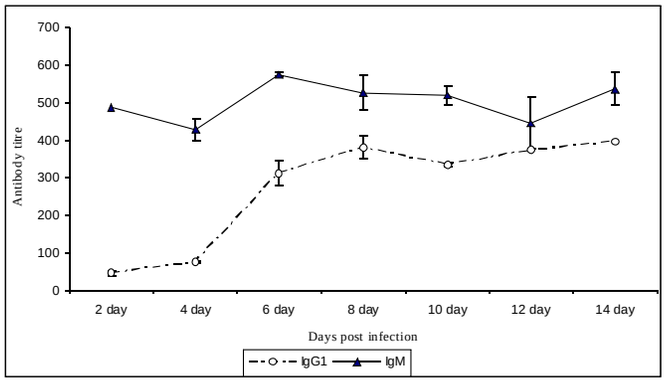


Fig. 13. IgM and IgG1 titres from pooled sera of mice subjected to adoptive transfer with JEV immune splenocytes, collected at different time points post infection.

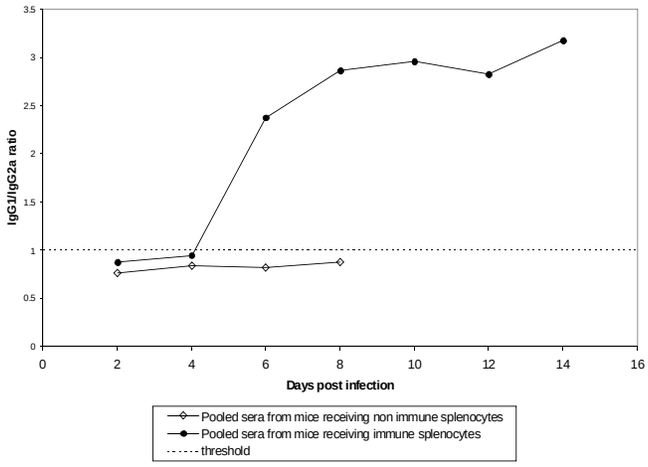


Fig. 14. IgG1/IgG2a ratios from pooled sera of mice receiving either JEV non-immune or immune splenocytes.

Differential expression of genes in mouse brain infected with JE virus.

SM Biswas, Rashmi Singh, V Vipat, MM Gore, D Ghosh

Our aim was to identify key molecules involved in the early response to JE infection in the brains of 14 day old mice. An extraneural route of infection was employed as it mimics the conditions of natural infection in man. Using the Discovery Mouse Chip, specific for 381 genes/probes, we identified 43 upregulated and 65 downregulated genes, whose expressions were modulated early during JE infection. Validation of the microarray data generated was done using Real time Sybr green RT PCR and RT PCR on a small subset of these genes.

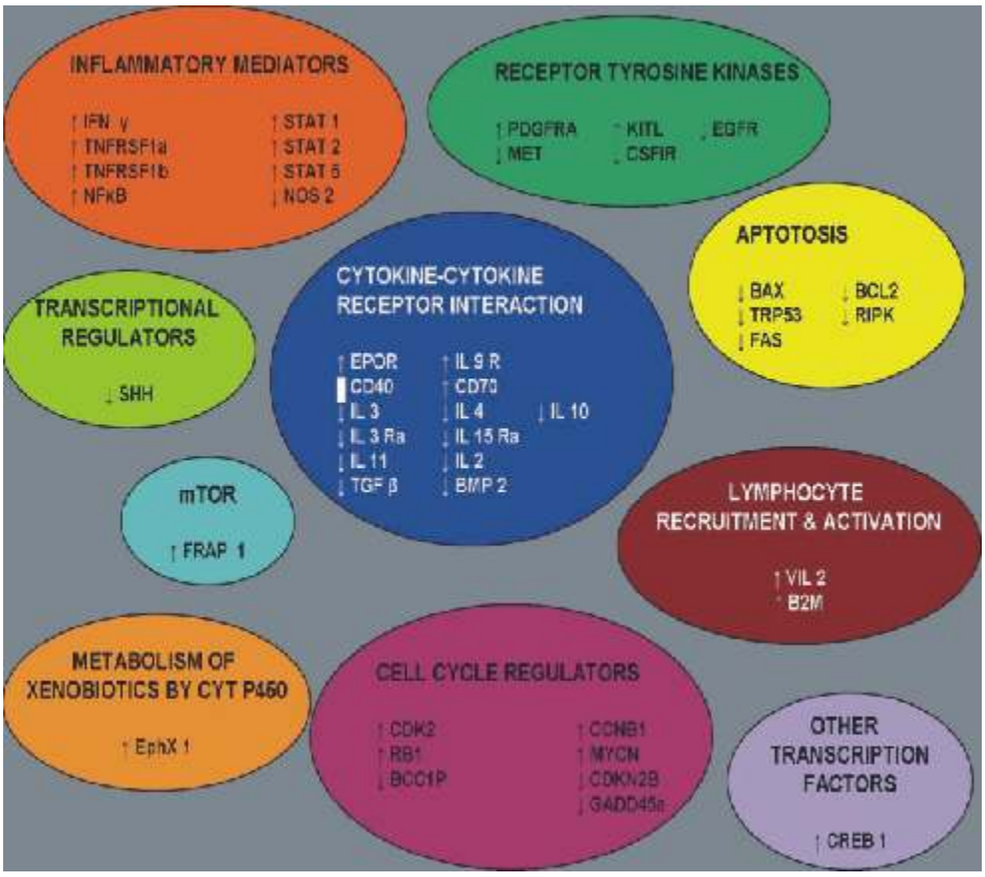


Fig.15. Grouping of the genes up and down-regulated in JEV infected mouse brain, upon microarray analysis, into functional groups.

Table.1. In the microarray analysis, 43 genes were upregulated out of 381 probes, of which 23 genes of our interest are represented in the table along with corresponding fold changes.

UPREGULATED GENES

GenBank Accession No.	Gene Name	Fold Change	GenBank Accession No.	Gene Name	Fold Change
NM_009283	STAT 1	20.436	NM_020009	FRAP1	38.791
AF187231	STAT 2	9.650	NM_008374	IL9R	14.09
NM_011488	STAT 5a	2.447	NM_009029	RB1	10.59
K00083	IFN-γ	18.354	NM_010301	GNA11	2.165
NM_011609	TNFR1	2.291	NM_011611	CD40	8.515
NM_011610	TNFR2	1.339	NM_011617	CD70	3.311
NM_009735	B2M	10.189	NM_009510	VIL-2	3.167
NM_008689	NFkB1	4.31	NM_016756	CDK 2	1.164
M95106	CREB 1	17.787	X64713	CCNB 1 (Cyclin B1)	1.407
NM_010149	EPOR	2.396	NM_008709	MYCN	4.636
NM_011058	PDGFRA	2.493	NM_021274	CXCL10	1.356
NM_013598	KITL	1.513			

Table.2. In the microarray analysis, 65 genes were downregulated out of 381 probes, of which 27 genes of our interest are represented in the table along with corresponding fold changes.

DOWNREGULATED GENES

GenBank Accession No.	Gene Name	Fold Change	GenBank Accession No.	Gene Name	Fold Change
NM_007527	BAX	0.113	AK010684	BCCIP (p21binding)	0.409
NM_007987	FAS	0.189	NM_011640	TRP53	0.683
NM_009741	BCL2	0.315	U41504	IL2	0.318
NM_009068	RIPK1	0.114	NM_010556	IL3	0.202
NM_007912	EGFR	0.1761	NM_008369	IL3Ra	0.8325
NM_007779	CSF1R	0.1639	NM_021283	IL4	0.326
NM_022994	DAP3	0.218	NM_010548	IL10	0.004
NM_009397	TNFAIP3	0.148	NM_010549	Il11RA1	0.236
NM_009424	TRAF6	0.613	NM_008350	IL11	0.540
NM_010730	ANXA1	0.599	NM_008354	IL12RB2	0.0630
NM_009367	TGFB 2	0.239	S80963	IL13RA1	0.5282
X76290	SHH	0.258	NM_008358	IL15Ra	0.542
NM_007836	GADD45a	0.213	NM_010927	NOS2	0.808
NM_007670	CDKN2b (p15)	0.6535			

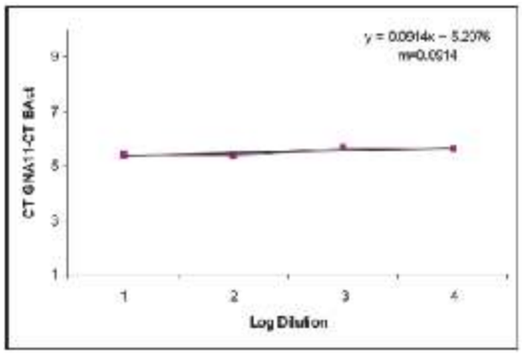


Fig.16. Calculation of relative SYBR Green PCR efficiency using serial 10 fold dilutions of the reference gene, í-actin and a target gene, GNA-1 1. A plot of the ?Ct versus log dilution gave a slope, m< 0.1, reflecting optimal PCR efficiency for both genes.

Table.3. Real Time SYBR Green data showing fold up regulation of genes from brains of mice on the 3rd day post infection as compared to sham-inoculated controls. Standard deviation for each gene value is indicated. Data represents an average of 4 datasets, each assay being performed with a pool of 2 mice brains. For an individual experiment, each gene was assayed in triplicates.

Up regulated Genes

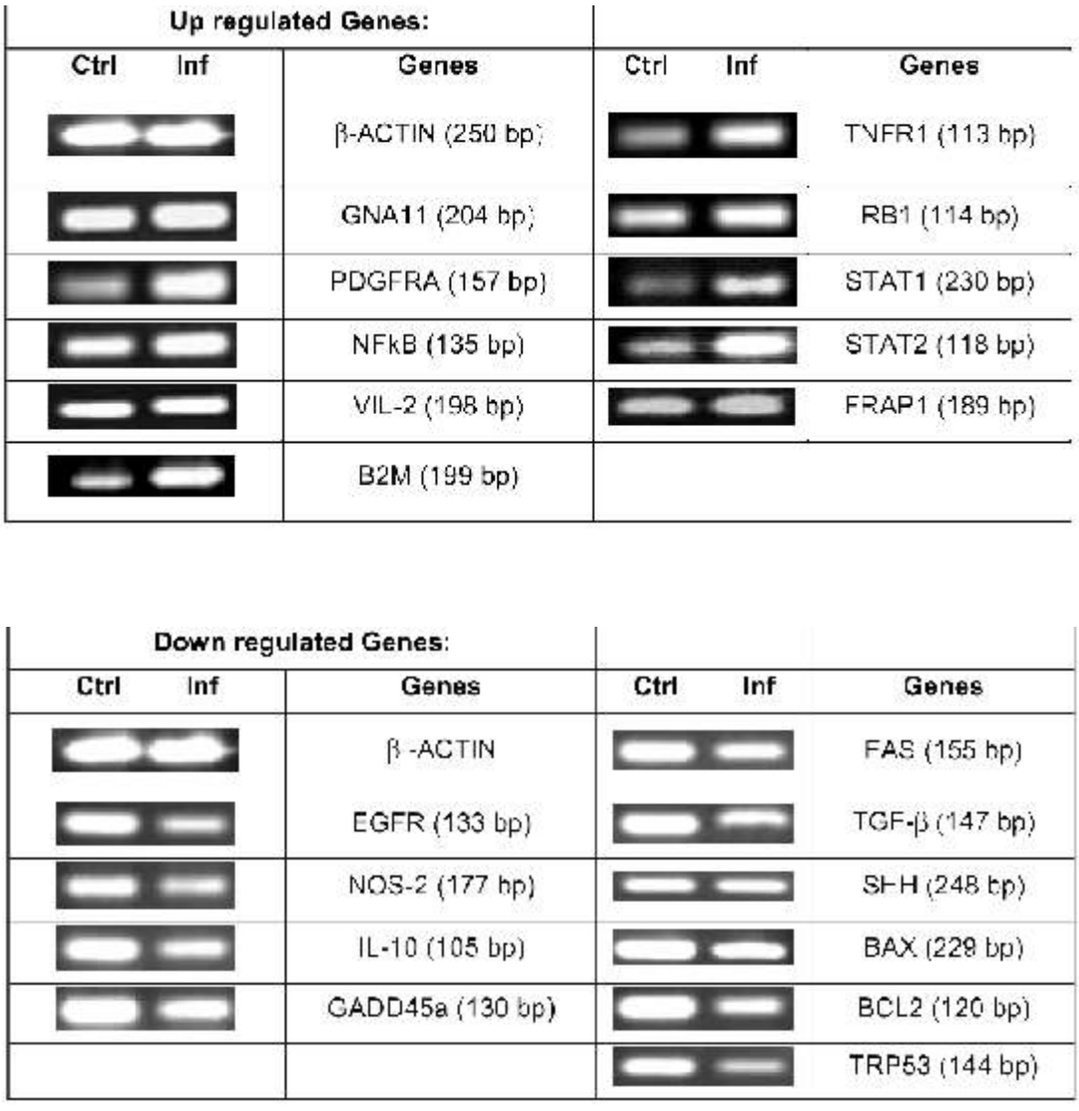
Gene Name	Fold Change (2-ΔΔCT)	StDev
GNA-11	1.227945	±0.157893
PDGFRA	1.137266	±0.144667
B2M	2.982893	±1.011186
VIL-2	1.484733	±0.487663
NF-kB	1.529815	±0.225173
TNFR1	1.209497	±0.16471
RB1	1.48855	±0.326664
STAT1	13.51539	±6.348694
STAT2	3.618133	±0.788336
FRAP1	0.957407	±0.26452

Table.4. Real Time SYBR Green data showing fold down regulation of genes from brains of mice on the 3rd day post infection as compared to sham-inoculated controls. Standard deviation for each gene value is indicated. Data represents an average of 4 datasets, each assay being performed with a pool of 2 mice brains. For an individual experiment, each gene was assayed in triplicates.

Down regulated Genes

Gene Name	Fold Change (2-ΔΔCT)	StDev
EGFR	0.5	±0.141421
NOS2	0.576874	±0.380064
IL-10	0.322266	±0.011054
GADD45a	0.925169	±0.341354
FAS	0.881044	±0.105838
TGF-β	0.691948	±0.390401
SHH	1.37	±0.04
BAX	0.700355	±0.016994
BCL2	0.873903	±0.335566
TRP53	0.816196	±0.0884

Fig.17. Up regulated and down regulated genes.



Post marketing study to evaluate the safety and immunogenicity of a single dose of JE vaccine at Burdwan, West Bengal

MM Gore, VM Ayachit, D Gangale, GN Sapkal

Background

In Burdwan West Bengal, 361 children were vaccinated with SA 14-14-2 vaccine in June 2006. Blood samples were collected on day 0, 28, 6 months and one year after vaccination. Sera were separated at the site and sent to NIV, for testing of neutralizing antibody response to Indian strain of JE (057434), and Beijing strain (Thailand) of JE virus. Serum virus neutralizing antibody titers were determined by carrying out PRNT with above named viruses. All the samples were stored at -20°C at NIV.

1. Day 0 samples

In order to assess the background immunity to JE and WN viruses in the children from Burdwan region prevaccination sera were tested for neutralizing antibodies against JE (Indian and Beijing) strains and WN viruses. Following results have been obtained.

Prevaccination N'Abs against JE strain from		
	India	Beijing
Total tested	345	345
Positive >10	159	87
% positive	46.08	25.29
Mean Ab titer	17.81	11.89

Individuals having <10 N'Ab titer on day 0 were grouped together while those with day o N'Ab titer >10 were grouped separately. Virus neutralizing antibody titers were determined after 28 days, 6 months and 1 year following the single dose of JE vaccine. Results obtained using Indian JE (057434) and Beijing JE virus are as follows:

Table. 1. Virus neutralizing antibody titers in 363 vaccinees tested against Indian JE virus strain 057434.

Days Post Vaccination	0 day	28 day	6 months	1 year
Group I: Individuals negative for anti JEV antibodies on day 0 (N= 193)				
Responders (%)		123/190 (64.74)	81/190 (42.63)	63/185 (33.68)
Geo Mean Titer	8.06 ± 1.31	21.10 ± 81.74	11.77 ± 129.11	9.01 ± 26.97
Group II: Individuals positive for anti JEV antibodies on day 0 (N= 167)				
Responders (%)		136/159 (85.53)	117/162 (72.22)	88/161 (54.66)
Geo Mean liter	24.59 ± 99.46	102.25 ± 2721.80	36.18 ± 286.03	22.26 ± 127.60
Total of Group I & II				
Responders (%)		259/349 (74.21)	198/352 (56.25)	152/351 (43.30)

Table. 2. Virus neutralizing antibody titers in 363 vaccinees tested against JE virus strain Beijing.

Days Post Vaccination	0 day	28 day	6 months	1 year
Group I: Individuals negative for anti JEV antibodies on day 0 (N= 262)				
Responders (%)		77/252 (30.56)	40/255 (15.69)	21/256 (8.20)
Geo Mean Titer	7.16 + 1.19	9.28 + 43.53	7.23+ 34.64	6.33 - 17.94
Group II: Individuals positive for anti JEV antibodies on day 0 (N= 100)				
Responders (%)		72/96 (75.00)	56/97 (57.73)	53/96 (55.21)
Geo Mean titer	27.04 = 51.11	58.04 ± 372.41	25.13 ± 86.39	25.99 ± 116.59
Total of Group I & II				
Responders (%)		149/348 (42.82)	96/352 (27.27)	74/352 (21.02)

A prospective open-label, uncontrolled, single-centre, evaluation of viraemia in healthy flavivirus seronegative adults after primary vaccination with JE SA14-14-2 live attenuated vaccine (JEATTE/IND/01- PUNE)

MM Gore, VS Padbidri, VP Bondre, GN Sapkal, PV Fulmali, V. Shankaraman, G Jogdand, Y Rajmane, VM Ayachit, D. Gangale

Objectives

Primary objective

- To determine levels of viraemia after administration of a single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine in adult subjects between days 1-8 and day 15.

Secondary objectives:

- To determine neutralizing antibody response at 30 days, 6 months and one year after administration of a single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine in adult subjects.
- To evaluate safety from the time of administration of a single dose of live attenuated SA14-14-2 Japanese encephalitis vaccine in adult subjects till one year.

1. Enrolment procedure:

The steps enumerated in the Protocol were followed for recruitment. The enrolment commenced at the site on 28 May 2007 and ended on 01 Feb 2008. A total of 287 people between the age of 18 and 40 years and both genders, were screened.

The subjects were first screened to see if they fulfilled the Inclusion and Exclusion criteria. They were then explained the project and their participation. They were also provided with the Patient Information Sheet and Informed Consent Form. After the subject signed the Informed Consent Form the clinical examination was carried out and his/her blood sample was obtained to determine the antibody status for Japanese encephalitis (JE), West Nile (WN) and Dengue (DEN) antibodies by performing the HI test. Values above 1:10 were considered as positive. The sera of those subjects who had HI titres of $\leq$ 1:10 were further tested in PRNT. Here also, the cut off point was a value above 1:10. Subjects who had values of $\leq$1:10 in both the tests were eligible to receive the vaccine.

The eligible vaccinees were then bled again and bio chemical/haematological tests performed, along with ELISA tests for HIV-1, Hepatitis B virus surface antigen and Hepatitis C virus. Urinalysis was also carried out in female vaccinees for routine and pregnancy tests. All these procedures were carried out during Visits 1 and 2.

On the day of vaccination (Visit 3) the subjects were given the Informed Consent Form for perusal and signature. Physical examination and urinalysis was carried out. They were assigned to one of the 3 groups of vaccinees. After administration of vaccine, they were kept under observation for half an hour. They were then provided with a clinical thermometer, measure tape and Daily Diary Card and explained how to fill it up.

2. Vaccinee groups:

There were three groups for the study.

Group 0 consisted of 12 vaccinees. They were required to report everyday, from Day 1 to Day 7, Post vaccination. (Visit 4 - Visit 7)

Group 1 consisted of 12 vaccinees. They were required to come on alternate days on odd days - Day 1, Day 3, Day 5 and Day 8 post vaccination (Visit 4 - Visit 7)

Group 2 consisted of 11 vaccinees. They were required to come on alternate days on even days - Day 2, Day 4, Day 6 and Day 8 post vaccination (Visit 4 - Visit 7)

The first batch of vaccinees was administered the dose on 26 June 2007. While the last batch was vaccinated on 18 Feb 2008.

3. Severe adverse events:

There was only one severe adverse event (SAE) reported so far, in the study. DTB, a male 20 years of age, Enrolment No. 019 from Group 1, received the vaccine on 30 Aug 2007. On 24 Nov 2007 he had moderate fever with chills (101^0) burning of eyes and giddiness, for which he was hospitalised on 27 Nov 2007 He was given symptomatic treatment and anti malarial therapy. The diagnosis was "viral fever with conjunctivitis". He recovered rapidly and was discharged on 30 Nov 2007. Recovery was complete and without any sequelae.

As per the Investigator the SAE was NOT RELATED to the vaccine.

The SAE was reported to the Local Safety Monitor by telephone on the afternoon of 30 Nov 2007. This was followed by sending her all the relevant information and proforma by fax on 01 Dec 2007.

Adverse events (AEs):

Several AEs were reported, which are summarized as below in the table.

Table. Summary of adverse events in vaccinees.

Sr. No.	Enrol No.	Vaccinee Group	Nature of AE
1	004	Gr. O	Headache
2	006	Gr. O	Headache, Weakness
3	008	Gr. O	Rhinitis, Pharyngitis Came with C/o generalised itching with dryness and tenderness in rt. hypochondriac region. Clinically there were no signs and USG abdomen was not significant. His SGOT (Normal upto 30 Units/L) and SGPT (Normal upto 40 Units /L) were found to be raised during visit 8 - SGOT 53 units /L and SGPT 101 Units/L. Clinically, these were not found to be significant
4	010	Gr. O	Headache
5	014	Gr. 1	This vaccinee had no complaints but his SGOT and SGPT levels were found to be raised Visit SGOT SGPT V2 49 73 V8 114 183 These were not clinically significant
6	015	Gr. 1	This vaccinee complained of weakness and loss of power in the left hand on 1/10/07. He was referred to the Neurologist. He was diagnosed to have Lt. radial neuropathy and advised physiotherapy and vitamins.
7	029	Gr. 2	Headache

All these AEs were not related to the JE vaccine administration.

4. Viremia in vaccinees

Level of Viremia was tested in vaccinees on different days as mentioned above. The sera were separated and titrated on BHK cells, which are capable of supporting SA 14-14-2 virus growth. In addition virus copy number was determined by Real Time PCR assay on the whole blood.

It was observed that only one volunteer Enrolment No. 002 showed detectable copy numbers on the 8th day of vaccination. (242 copies /mL). All other subjects did not show any detectable virus level in either serum or whole blood.

5. Antibody response to vaccination.

Blood samples were collected on 0, and 30 day after vaccination. Analysis of antibody titers obtained 30 days after vaccination revealed that out of 35 subjects 9 did not sero convert (Sero-conversion in 74.28%). Titers are in the range of 10.43- 133.03. Assay for antibody titers in serum samples collected after six months after vaccination has been completed in 20 subjects. Only 1 out of 20 subjects has shown antibody titers >10.0

Further work

Samples would be collected for testing the antibody titers after six months and one year for the remaining vaccinees. The clinical assessment of the subjects at the end of this period would be analysed. The data would be analysed by the Data Management Team of the study. The report would be then submitted.

Selective Expression of Recombinant Viral Protein in Immunocompetent Cells

MF Ahsan, MM Gore

Background

In conventional DNA vaccines the expression are controlled by a nonspecific (CMV) promoter making it possible to express the protein in all the cells, which get transfected including that of nonprofessional APCs. This might affect the overall intensity and longevity of immune response *in-vivo*. Hence, the approach is to express the antigen only in professional APCs, this could be achieved by using cell specific promoters.

Objective

Cloning and Expression of structural genes of Japanese Encephalitis Virus under the control of immune cells specific promoter. Study immune response and protection *in-vivo* when those recombinants are used as a plasmid vaccine.

Work done

Employing Reverse Transcriptase PCR, E-gene with and without transmembrane sequence of Japanese encephalitis virus (strain - 733913) was amplified. Amplicons were cloned into pGEM T Easy vector and transformed to JM 109 competent cells. Gel of isolated plasmid of ligated product of pGEMT (3KB) and PCR product of complete and truncated E

JEV E-gene (with and without transmembrane sequences) from TA Cloned pGEM T Easy vector was excised and cloned in pAcGFP1-N1 expression vector at EcoR I site and transformed into JM 109 competent cell. Recombinant vector was isolated by plasmid mini prep method and JEV E gene was confirmed by restriction digestion analysis. By sequencing it was observed that the start and stop codon is in-frame. Both the codons were

included in primers at the time of primer design. Digestion pattern of both the clones (Complete and Truncated E) when digested with two different Restriction enzymes as a confirmation of insert. The clones were further confirmed through sequencing.

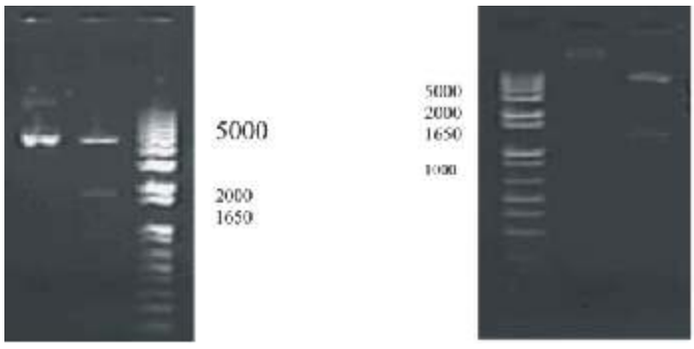


Fig.18. Cloning of E gene pAcGFP1-N1 vector.

1	Undigested vector of 4.7 KB	1	1 Kb+ DNA Ladder
2	Clones (complete E protein) digested with SacI fragments size of 4999 & 1812 bp.	2	Undigested cloned vector of 7 KB
3	1 Kb+ DNA Ladder	3	Clones (Truncated E protein) digested with Hpa I fragments size of 1263 & 5242 nt.

Protocol was standardized with Lipofectamine 2000 for the Transfection of plasmid (pAcGFP1-N1) with CMV as a promoter in Vero and RAW 264.7 cell lines, Opti-MEM was used as a medium. After 24 hours of post transfection, cells were observed for fluorescence under fluorescent microscope. As a negative control, cells without plasmid were transfected with Lipofectamine 2000. For macrophage specific promoters, macrophage specific markers have been selected and the sequence retrieved from GenBank. Primer would be designed using Primer Express version 2.0 and various online available tools (Primer3, Oligo-analyzer, etc.)

A B

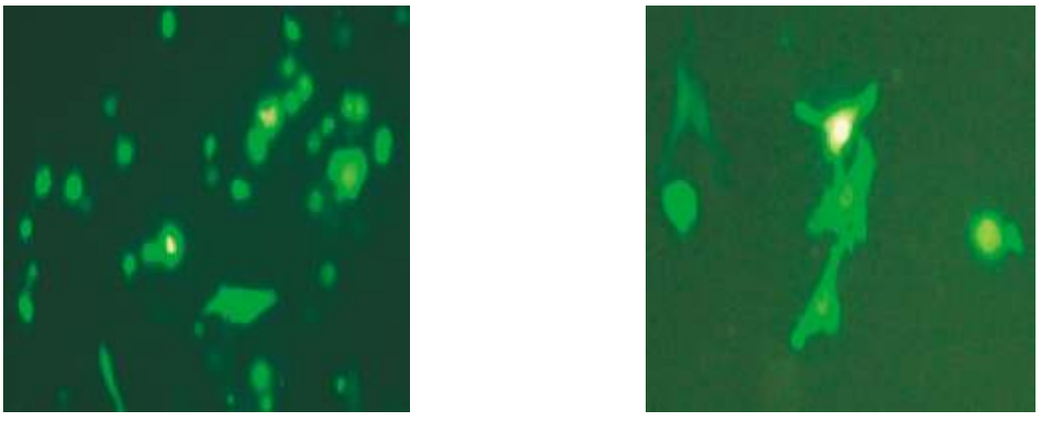


Fig.19. Expression of GFP post transfection in (A) Vero cells and (B) RAW 264.7 cell line

Findings

Cloning of truncated and complete envelope protein of JEV has been achieved. Standardization of transfection using Lipofectamine 2000 in Vero and RAW 264.7 cells has been achieved.

Development of molecular techniques for the rapid detection of agents infecting the central nervous system

VP Bondre, PV Fulmali, GN Sapkal, V Shakaraman, and MM Gore.

Background

Acute viral encephalitis is known to be caused by a wide range of viruses either in sporadic or in outbreak forms. Globally identified viral etiological agents include Herpes, Entero, Alpha, Influenza A, Rabies, Human Immunodeficiency and Flaviviruses. In India, Japanese encephalitis (JE) and West Nile viruses co-exist in many areas. Some of the cases go undiagnosed because of the unavailability of the sensitive diagnostic techniques. Hence, there is need to developed highly sensitive diagnostic methods. As a first step flavivirus specific semi nested RT PCR has been developed.

Objective

- Development of a highly sensitive semi nested RT-PCR based diagnosis for Flavivirus detection.

Work done

Flavivirus diagnosis

A one step reverse transcription polymerase chain reaction (RT-PCR) followed by seminested PCR was developed for Flavivirus detection and standardized using laboratory strain of titrated Japanese Encephalitis, West Nile Virus and Dengue 2 and Bagaza virus. The other viruses viz, Chikungunya (Alphavirus) and Chandipura (Rhabdovirus), negative human clinical specimens and normal vector mosquitoes were also included to check for non specific amplification. Sensitivity of RT-PCR and semi-nested PCR was determined by comparing the endpoint dilution of plaque titrated different flaviviruses stocks grown in cell culture and infected mouse brain suspensions. The sensitivity RT-PCR using serially diluted WN (68856) virus (7.3 $\log_{10} LD_{50}$/ml) was determined.

Findings

The one step RT-PCR specifically detects WNV and does not cross-react with other viruses (Alphaviruses and Rhabdoviruses), however, its sensitivity to detect titrated WNV virus falls between 100-1000 plaque-forming units (pfu). The semi nested PCR of products amplified in one step RT-PCR specifically detected 1-10 pfu of WNV virus. Additionally, the sequence confirmation of WNV (68856) of semi nested PCR amplified product has confirmed the specificity of our semi nested PCR. The assay is being used for diagnosis of specimens from encephalitis cases.

Publications

- Bondre VP, Jadi RS, Mishra AC, Yergolkar PN, Arankalle VA. West Nile virus isolates from India: evidence for a distinct genetic lineage. **J Gen Virol**, 2007; 2007 Mar; 88(Pt 3): 875-84.
- Dewasthaly SS, Bhonde GS, Shankarraman V, Biswas SM, Ayachit VM, Gore MM. Chimeric T helper-B cell peptides induce protective response against Japanese encephalitis virus in mice. **Protein Pept Lett,** 2007; 14 (6): 543-51.
- Sapkal GN, Wairagkar NS, Ayachit VM, Bondre VP, Gore MM. Detection and isolation of Japanese encephalitis virus from blood clots collected during the acute phase of infection. **Am J Trop Med Hyg,** 2007 Dec; 77(6): 1139-45

Workshops / Conferences / Seminar / Meetings attended

MM Gore

- Oral presentation on "Differential dominance of $CD8^+$ and $CD4^+$ cell population in protection from Japanese encephalitis and West Nile virus infection in mice" in International conference organized by Indian Virological Society, New Delhi at PUSA campus during December 11 -13, 2007.

VP Bondre

- Oral presentation on "Molecular epidemiology of Japanese encephalitis virus in India: Scenario during last fifty years (1956 2006)" in International conference organized by Indian Virological Society, New Delhi at PUSA campus during December 11 -13, 2007.
- Invited lectures "Epidemiology of Japanese encephalitis: Global and National perspectives" in State level Workshop on Japanese encephalitis, organized by National Vector Borne Disease Control Programme, Govt. of India, the Department of Family Welfare and Arogyakeralam at Tiruvananthapuram (Kerala) on March, 12 -14, 2008.
- Invited lectures "Viral agents causing encephalitis" in State level Workshop on Japanese encephalitis, organized by National Vector Borne Disease Control Programme, Govt. of India, the Department of Family Welfare and Arogyakeralam at Tiruvananthapuram (Kerala) on March 12 -14, 2008.

GN Sapkal

- Training course in Flowcytrometry at New Delhi during July 2-5, 2007.

Patent

Indian, Vietnam, Korea, US Patent granted to Gore, M. M., Dewasthaly, S. S., Kolaskar, A. S., Kulkarni-Kale, U. D. (April, 2007). Chimeric T helper B-cell peptide as a vaccine for flaviviruses.

Dengue

Dengue

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4 Dengue

- Disease burden for dengue (Establishing the base line data required for designing a study)
- Evaluation of DENV- NS1 Ag ELISA for early diagnosis of the disease
- Evolution of dengue viruses in the country
- The role of host/viral factors in dengue immunopathogenesis
- Interactions of DENV with cellular organelles during viral morphogenesis
- Studies on the effect of dengue viruses on *in-vitro* hematopoiesis and demo static physiology of vasenlar endotrelial cells
- siRNAs an antiviral tool against dengue virus
- Additional Studies
  Generation of HIV-1 subtype C based DNA vaccine candidate & assessment of prime boost immunization strategy in mouse model

Disease burden for dengue (Establishing the base line data required for designing a study on disease burden)

PS Shah, Anand Singh, Rupali Bachal, Asha Bhagat, Cecilia D.

Dengue is endemic to Pune and several other cities in Maharashtra. It is of major relevance to disease management that the cost of the disease to public health is established. Before designing a study on disease burden we have addressed the following questions to generate base line data for dengue in Pune. Do the intensity of outbreaks, the demography, the severity of the disease, the dominating virus serotype and the seasonal pattern change from year to year? We have taken up the study in collaboration with two major hospitals, KEM, Pune, YCMH, Pimpri and five clinics spread out in the city and will be covering a period of five years from 2005 to 2009. The data that has been generated each year has been reported in the annual reports and will be collated at the end of the five year period.

Objectives

- Determine the proportion of clinically suspected dengue cases with confirmed DENV aetiology.
- Categorize the confirmed dengue cases according to the disease severity in Pune.
- Determine the seasonal incidence of dengue
- Determine the serotype of the circulating dengue virus

Work done

A study of the febrile cases referred by the clinicians in and around Pune revealed that the incidence of dengue this year was very high compared to 2006. A total of 882 samples were tested from suspected dengue cases in Pune during Jan 07-Dec 07. Of these, 332 (37%) sera were positive for dengue-specific IgM antibodies. **Fig 1** shows the categorization of the confirmed dengue cases in 2007. DF cases (81%) were predominant with 29% cases showing thrombocytopenia. Only 19% of the cases were DHF with 6% belonging to DHFI, 12% to DHFII and 1% to DHFIII, there were no DHFIV cases observed in the cases we investigated.

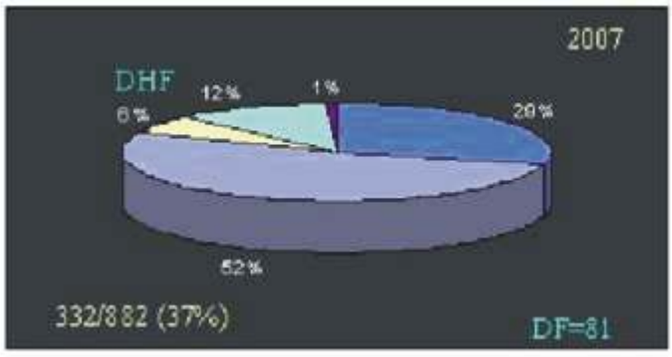


Fig. 1. The confirmed dengue cases were categorized according to WHO classification

The largest group that was affected was the 20-30 yr age group. The males were better represented than females. The largest number of cases was observed in September. Three seroytpes DENV-1, 2 and 3 were found to be circulating.

We also test samples from different hospitals and PHCs across the state of Maharashtra. Mumbai had 546 suspected cases of which 48% were confirmed. The other cities with 70-100 cases with a positivity ranging between 20-30% were Nashik, Solapur, Sangli, Washim and Nagpur. Altogether Maharashtra had 1336 suspected

cases of which 439 were DEN-IgM positive (32.9%) from Jan-November 2007. A rural outbreak in a village of Bhor (Pune district) was investigated in June 2007. Thirty one cases were investigated of which 13 were IgM positive and 15 were PCR positive resulting in a 74% confirmed cases. DENV-1 was the predominant serotype and all cases were DF. In March 2008, another rural outbreak in Gojubavi, a village in Baramati was investigated. Of 78 suspected cases, 48 were IgM positive and four were PCR positive, thus resulting in 66% positivity. The predominant serotype was once again DENV-1 and like Bhor all cases were DF. Therefore DENV-1 seems predominant in rural Pune causing less severe disease.

Future Plans

Similar studies will be carried out in the next year and more aggressive investigations will spearheaded in periurban areas. At the end of five years the data will be collated and analyzed to achieve the objectives mentioned above.

Evaluation of DENV- NS1 Ag ELISA for early diagnosis of the disease

PS Shah, Anand Singh, Cecilia D

Detection of dengue cases before seroconversion, which is important for patient management and determination of disease incidence, remains a challenge. We have been carrying out RT-PCR for detection of viral RNA in the early phase of infection. Another option for the early diagnosis of DENV-infection is the detection of NS1 Ag in patient sera. Commercially available Platelia Dengue NS1 Ag assay kit was evaluated for its ability to detect early cases in comparison to RT-PCR.

Objective

- Assess the use of Platelia Dengue NS1 Antigen Capture ELISA for early detection of dengue cases.

Work done

Initially 41 dengue samples, known to be positive by Real time RT-PCR were used to evaluate the NS1 ELISA in comparison with multiplex RT-PCR. NS1 antigen was detected in 30 of 41(73%) samples while dengue RNA could be detected by RT-PCR in 27(66%) samples. The NS1 ELISA was then tested with 39 sera from suspected dengue cases, which showed IgM in 14 (36%) samples. Out of 39 samples, 13 (33%) were positive by NS1 ELISA and 21 (54%) turned out to be positive by RT-PCR **(Table 1)**.

Table 1. Comparison of NS1 Ag ELISA with RT PCR in the detection of dengue cases

Cases tested	No. pos for NS-1 Ag (%)	No. pos by RT-PCR (%)
Known cases (Real time PCR pos), n=41	30 (73)	27 (66)
Suspected cases, n=39	13 (33.3)	21 (53.8)
Total n=80	46 (57.5)	48 (60)

The RT-PCR assay detected more cases (29) than NS/ELISA (23) before seroconversion (n=46) while the NS1 Ag ELISA detected more cases (23) than RT-PCR (15) after seroconversion. Considering the ease of the NS1 Ag ELISA and the smaller volume of sample required, the test could be used for early diagnosis of the disease.

Future plans

The NS1 antigen based ELISA will be applied to our investigations in rural areas.

Evolution of dengue viruses in the country

JA Pawar, Cecilia D

Phylogenetic analysis of the DENV circulating in India is being pursued since 2003 to understand the evolution of dengue viruses in the country. We had earlier reported the genotypes of the Indian strains of DENV-1, 2, 3 and 4 circulating in the country during the last fifty years. To further understand the evolution and dispersion of the virus in the country, full genome sequencing was undertaken.

Objectives

- Sequencing of the full genome of DENV strains of all four serotypes.
- Analysis of the sequence- data based on the genetic diversity of the different genes, selection pressures and possible recombination sites.

Work Done

Virus strains representing each decade were selected. The full genome of 15 strains of the four serotypes has been sequenced. The sequences are being analyzed for phylogeny based on whole genome, genetic diversity of the different genes, the selection pressures and possible recombination sites.

Seven genes C, prM, E, NS1, NS2a, NS2b and NS3 of two strains of DEN-4 which were 17 years apart were compared and it was found that the prM, NS2A, NS2B, NS3 genes were strongly conserved Capsid, E gene and NS1 were less conserved.

Future plans

Analysis of the whole genome sequences generated will be completed and a few more strains of DENV-3 will be sequenced.

The role of host/viral factors in dengue immunopathogenesis

Debargh Dutta, Cecilia D

Both host and virus factors are important in the development of severe dengue disease. So far we had shown that both arms of the immune response, the cytokine and antibody response were differentially regulated in DF and DHF cases. This time we used the C57BL/6 mice to study the immunoregulation by infected DCs in mice.

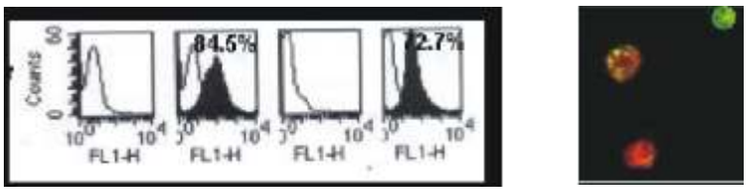
Objectives

- Assess the susceptibility of DCs myeloid (DC1) and plasmacytoid (DC2) to DENV infection.
- Study the effect of DENV infection on the two populations of DCs.
- Determine the effect of dengue-infected DC1 and DC2 on T cells

Work done

The two populations of DCs DC1 and DC2, derived from the bone marrow of C57BL/6 mice were found to be

susceptible to DENV infection. However, the virus was found to regulate the two populations differently. There was increased expression of co-stimulatory and regulatory molecules on DENV infected DC1 as compared to DENV infected DC2. Inoculation of mice with infected DC2 showed generation of regulatory T cells (Treg cells) in the spleen, whereas, inoculation with infected DC1 induced a strong proliferative response in the spleen. $CD4^+$ T cell from DENV immunized mice upon secondary stimulation with infected DC1 showed marked decrease in their proliferation suggesting activation-induced cell death. No difference in T cell proliferation was noticed when cells were restimulated with infected DC2.



A B

Fig.2. Purified populations of DC1 and DC2 infected with DENV-2. A) Shows the flowcytometric analysis and B) shows the presence of virus antigen in DCs (green) by IFA

Future plans

The differential effect of dengue virus on the two populations of DCs and their immunostimulatory potential in mice was an interesting finding and it will be interesting to see whether the same phenomenon is observed with human DCs.

Interactions of DENV with cellular organelles during viral morphogenesis

Samatha Sripada, Cecilia D.

Majority of viruses use cytoskeletal components of the host cells to gain entry into cells, replicate and spread to adjacent uninfected cells. Our earlier studies on dengue morphogenesis demonstrated that all three cytoskeletal elements of fibroblast cells are modified upon virus infection. This year the detailed interaction of DENV with microfilaments was investigated. During entry DENV was found to induce the *de novo* formation of filopodia facilitating entry of virus particles. In normal cells de novo actin polymerization is regulated by the Arp2/3 complex. We therefore investigated the possible interaction of DENV with Arp2/3 complex during virus entry.

Objectives

- Analysis of changes induced in microfilament structure during entry, maturation and egress of DENV-2.
- Investigating the role of Arp2/3 complex in nucleation of actin microfilaments during entry.

Work Done

PS (porcine kidney fibroblast cells) were infected with DENV, fixed at various time points and stained for actin and DENV with fluorescent probes. For analyzing the early events of virus entry, surface staining of cells for DENV was carried out without fixing or permeabilization. The images were acquired on Zeiss LSM 510 confocal microscope. Virus was labeled with secondary antibody-TRITC (red), actin and Arp2/3 were labeled with probe-FITC (green). During the process of infection, within 15-30 min of virus addition to cells, Arp2/3, which showed cytoplasmic distribution in normal cells, was found to relocate to the cell periphery associated with virus **(Fig.3A)**. High magnification rendering of infected cells showed close association of DENV on the cell surface (concluded because cells were stained for virus without permeabilization) with Arp2/3 **(Fig.3B)**. This suggested that DENV-2

recruited Arp2/3 as the nucleating complex for inducing actin rich filopodia.

Ours is the first report to show the selective recruitment of Arp2/3 complex by a virus to cell surface thereby facilitating entry.

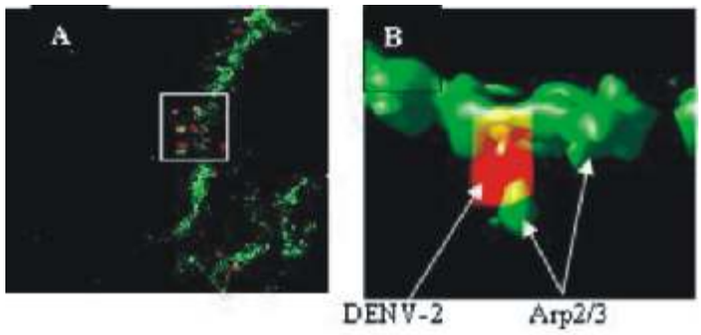


Fig. 3: Association of DENV-2 with Arp2/3 complex. Deconvolved images of DENV-2 infected cells showing the distribution of Arp2/3 complex (green) and viral antigen (red). (B is the area magnified from A)

Double labeling of DENV and actin at 15 min showed virus aligned along the filopodia (**Fig.4A)**. At 60 min p.i. there was thinning of the actin cortex **(Fig.4B)** which is characteristic of endocytic hotspots. Thus on binding to the cells DENV induced formation of filopodia and thinning of the cortex to facilitate entry.

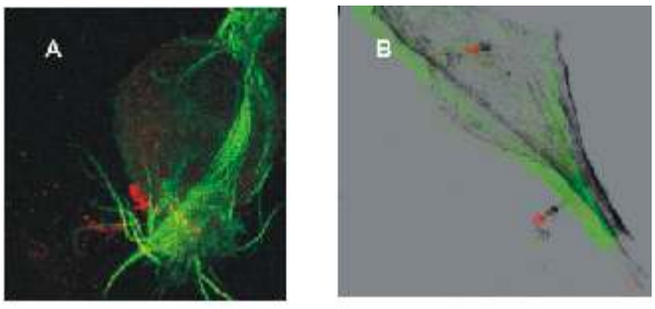


Fig.4: A) shows association of virus aggregates with filopodial extensions at 15 min p.i. ; B) Shows localization of DENV at the surface of the cell at 60 min p.i. DENV Infected cells were labeled for actin (green) and DENV (red).

Maturation phase of DENV was characterized by intact MF, which may be required for virus protein translation and assembly. However, there was no direct association between viral antigen and MF. **(Fig.5).**

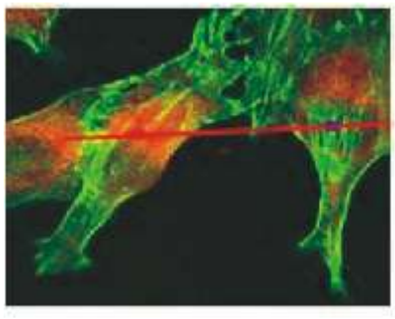


Fig. 5; Actin reorganization during DENV maturation. Infected cells were fixed at 48 h p.i. and double labeled for actin (green) and virus (red).

During egress, the virus induced the formation of thick peripheral bundles possibly a mechanism to propel mature virions out of the cell **(Fig.6)**. Inhibition of actin polymerization by treatment of cells with cytochalasin D at different time points pre and post infection resulted in decrease in virus yield as measured by PFU and real time PCR assays. The microfilament structure was thus required for all stages of virus replication.

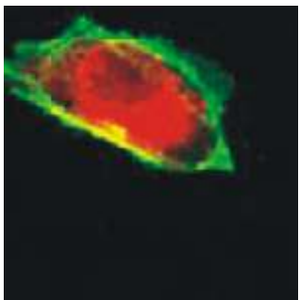


Fig.6; Actin reorganization during DENV egress. DENV-2 infected cells were fixed at 72 h p.i. and double labeled for actin (green) and virus (red).

Future plans

The role of the other cytoskeletal components in DENV replication will be investigated.

Studies on the effect of dengue viruses on in-vitro hematopoiesis and hemostatic physiology of vascular endothelial cells

A Basu, P Jain, SV Gaongodkar

This program was initiated in 2005 in collaboration with the National Institute of Immunohematology (ICMR) Mumbai and is extramurally funded by the Dept of Biotechnology Govt of India. The major objectives are to characterize at a molecular and cellular level, the mechanisms by which dengue viruses cause hematological dysfunctions-specially thrombocytopenia and related platelet disorders.

In continuation with the previous years work, the effect of dengue 2 virus on the in-vitro differentiation of hematpoietic progenitor stem cells was carried out to include multilineage differentiation including thrombopoiesis. The dengue 2 virus was shown to selectively inhibit human megakaryocyte growth and differentiation and induce apoptosis in early CD34+CD61+ megakaryocytic progenitors. [(1)] (Basu A et al 2008) Importantly, erythropoiesis remains unaffected and detailed studies on granulopoiesis are ongoing.

The major achievement in the second year of the project was the standardization of components of vascular biology. Human endothelial cells from HUVEC and primary lung microvascular beds were grown and characterized with an objective to study the effects of dengue virus induced alterations in vascular physiology and hemostatic properties. The complete envelope glycoprotein (E) and NS1 from dengue 2, dengue 3 virus and JEV have also been cloned into pcDNA3.1 for transfection studies in these cells. The optimization of efficient transfection of the endothelial cells with these constructs are currently undergoing.

siRNAs an antiviral tool against dengue virus

PS Shah, Guru Kumar KR and Cecilia D

Partial inhibition of virus replication in vitro by treatment of cells with chemically synthesized siRNAs as well as cloned siRNA sequences (shRNA) targeted against the 3'UTR of DENV genome was reported previously. To achieve complete inhibition of virus replication, we targeted two additional regions, the junction of core and prM and the NS3 gene. For sustained delivery of siRNA, plasmid based vector pSilencer H1 neo (Ambion) was used, which had an antibiotic resistance marker (neoR), useful in generating cell lines that stably express siRNAs.

Objective

- Cloning of the oligonucleotides representing the target siRNA sequences into pSilencer H1 neo vector.
- Testing the antiviral effect of 3'UTR, C-prM and NS3 shRNAs against DENV.

Work done

To select the potential siRNA targets, sequences of 11 Indian strains of DENV1, 2, 3 and DENV 4 serotypes of dengue viruses were aligned and analyzed. No continuous stretch of 21 nucletotides conserved in all four serotypes was found. Therefore, two sets of oligonucleotides representing C-prM conserved in DENV-1, 2 & 3 and NS-3 conserved in DENV-1, 3 & 4 were synthesized.

The oligonucleotides were cloned into the pSilencer-H1 Neo vector and two clones targeting CprM (pSil-CprM) and NS3 (pSilNS3) were generated. The tests to assess the antiviral effect have been standardized.

Future plans

The inhibitory effect of pSil-UTR, pSil- NS3, and pSil-CprM against different serotypes of DENV will be assessed. Stably transfected cells expressing siRNA will also be generated.

Additional Studies

Generation of HIV-1 subtype C based DNA vaccine candidate & assessment of prime boost immunization strategy in mouse model

(Extramural project RS 72, 06,494)

Srikant Tripathi, SM Mehendale, Madhuri Thakar, Smita Kulkarni, PS Shah, Cecilia D

Introduction

The development of a candidate vaccine for HIV-1 Clade C using the DNA prime protein boost vaccine approach was undertaken in collaboration with NARI. The aim was to generate recombinant pVax expressing the envelope, codon optimized envelope and gag genes and test their immunogenicity with and without immunomodulators in mice.

Objectives

- Evaluate the immunogenicity of HIV-1 Clade C envelope and gag genes with or without immunomodulators in mice.

Work Done

Three week old mice were immunized using 6 different constructs:

Native envelope (gp150), Codon optimized envelope, Codon optimized envelope with immuno-modulators, Native envelope (gp150) with immuno-modulators, Gag, Gag with immuno-modulators and control pVAX-1.

The sera samples were collected on 0, 3, 5 and 7 weeks post immunization and were assessed for antibodies against gp120 by ELISA. The best antibody response was seen with codon-optimized gp150 gene. The response was slightly improved with the addition of immunodulators.

Future plans

Further experiments are ongoing with improved vectors and schedules.

Publications

- Basu A, Jain P, Gangodkar SV, Shetty S, Ghosh K. Dengue 2 virus inhibits in-vitro megakaryocytic colony formation and induce apoptosis in thrombopoietin-inducible megakaryocytic differentiation from cord blood CD34+ cells **FEMS Immunology & Medical Microbiology** 2008: 53: 46-51

Presentations in the Conferences:

- Priyadarshini D and Cecilia.D. The levels of cytokines and neutralizing antibodies in dengue cases in India and its relevance to DHF. Oral presentation at the 13th International Congress of Immunology, Rio de Janeiro, Brazil, August 21-25, 2007.
- PS Shah, Harshad Patil and Cecilia.D siRNAs an antiviral tool against dengue. Oral presentation at Third Asian Regional Dengue Research Network Meeting Taipei, Taiwan, August 22- 24, 2007.
- Jayashri Pawar and Cecilia D. Molecular evolution of Dengue 1 in India. Third Asian Regional Dengue Research Network Meeting Taipei, Taiwan, August 22- 24, 2007.
- Samatha Sripada and Cecilia D. Significance of cytoskeleton in dengue virus replication. Frontiers in Cellular, Molecular and Developmental Biology, Dresden, Germany, September 1- 4, 2007.
- Anand Singh, PS Shah and Cecilia D Early detection of Dengue cases using NS1 antigen based ELISA. International Conference of Emerging and Reemerging Viral Diseases of Tropics and Sub-tropics, New Delhi, December 11-14, 2007.
- Guru Kumar KR., Priyadarshini D, JA Pawar, Asha Bhagat and Cecilia D. Detection and quantitation of Dengue viruses by Real Time PCR Method. International Conference of Emerging and Reemerging Viral Diseases of Tropics and Sub-tropics, New Delhi, December 11-14, 2007.

Workshops / Conferences / Seminar / Meetings attended

Cecilia D.

- Meeting of Health Minister of the States to review Chikungunya fever situation held at Vigyan Bhawan. DGHS, Ministry of Health and Family Welfare on June 21st, 2007.
- Invited to attend the Asia-Pacific Dengue Prevention Board meeting at Colombo, Sri Lanka, June 2007.
- International Workshop on Molecular Epidemiology and Immunology of Malaria and Other Vector Borne diseases at Jabalpur, 16-19 October 2007.
- Dr. Ananthanarayan Symposium at PGI, Chandigarh on 29 November 2007.
- 1st Meeting in March 2007 with Health Ministry of Kerala regarding the establishment of a Field Unit of NIV at Alappuzha, Kerala.

Chandipura Encephalitis

Chandipura Encephalitis

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5 Chandipura Encephalitis

- Analysis of host immune response against Chandipura virus in murine model
- Functional analysis of Chandipura virus recombinant proteins (M&N) and its utility in diagnostics and vaccine
- Chandipura virus encephalitis: The role of virus interaction with toll like receptor 4
- Cloning and expression of G-gene of Chandipura virus and evaluation of immunogenicity in mice
- Evaluation of siRNA in curing Chandipura virus infection *in-vitro* and *in-vivo*
- HLA Phenotypes and outcomes of Chandipura virus infection in Andhra Pradesh
- In-silico sequence and structure-based antigenic analysis of the G, N and M proteins of Chandipura virus

Analysis of host immune response against Chandipura virus in murine model

B Anukumar and AC Mishra

Age dependent susceptibility to Chandipura Virus (CHPV) was noticed in mice through all routes except intra cranial route of inoculation. Pathogenesis in adult mice through intra cranial inoculation was studied in this project.

Objective

- To study the immuno pathogenesis of CHPV in murine model

Work done

Pathogenesis in adult mice

Three groups of mice were inoculated with CHPV Blood Brain Barrier (BBB) through intracranial, intra nasal and intra venous (BBB Damage) route respectively. Mice were followed up to 96h PI and blood and brain was collected every 24h interval up to 96h PI. Virus titer in blood and brain, IgM, IgG kinetics, mortality pattern and level of CD4+, CD8+ and CD19+ cells in blood are the parameters studied. Detectable level of virus titer only noticed in blood at 24h PI in all the groups with various levels. Continuous replication of virus noticed in brain from 24h PI onwards invariably in all groups. Seroconversion noticed from 48h PI onwards but none of the mice switched into IgG type. All the mice died in intracranial as well as BBB damaged mice at 96h PI. No significant level of difference noticed in CD4+, CD8+ and CD19+ cell populations in these mice in all the PI hours tested. This experiment concluded that entry of virus in the brain is essential for CHP pathogenesis. Even though the virus reaching the brain through intra nasal route, the virus replication was restricted in some part of brain and no mortality observed.

Mechanism of reduction of immune cells in Chandipura infection

Reduction of CD4+ and CD8+ cells were noticed in Chandipura virus infection in both *in vivo* and *in vitro*. In this study role of Fas/Fas L in reduction was studied. *In vitro* infected RAW cells upregulated significant level of Fas L simultaneously down regulated Fas expression. Similarly splenocytes co cultured along with infected RAW cells also significantly upregulated the Fas/Fas L expression. Significant level of reduction in CD4+ cells noticed when the splenocytes cultured with supernatant from virus infected RAW cells. The supernatant contained significant level of TNFα, MCP-1 and IL-10. *In vivo*, the CD4+, CD8+ and CD14+ cells from CHP infected mice upregulated the Fas/ Fas L expression. The plasma from infected mice contains significant level of all pro inflammatory cytokines including TNFα. MCP-1, IL-10, IL-6, IL-12 and IFNγ. Purified CD4+ and CD8+ cells from infected mice were highly positive for Annexin V staining. Overall these experiments indicated that the reduction of CD4+ and CD8+ cells are regulated by expression of Fas/Fas L and secretion of proinflammatory cytokines

Immune status of CHP infected susceptible mice

Immune status of mice during infection was studied by lymphocyte proliferation assay using specific (inactivated antigen) and non specific (Con A and LPS) stimulators and IFNγ secretion assay. Antigen specific proliferation was only noticed in 48h PI, similarly LPS mediated proliferation was also noticed in 48h PI. Results based on Con A stimulation indicated that suppression of proliferation T cells in all post infective days. Significant level of IFNγ secreting CD4+ and CD8+ cells observed in 24h PI. At 48h PI only significant amount of CD4+ cells secreted IFNγ.

These experiments indicated that during infection mice undergone immune suppression due to various factors.

Functional analysis of Chandipura virus recombinant proteins (M&N) and its utility in diagnostics and vaccine

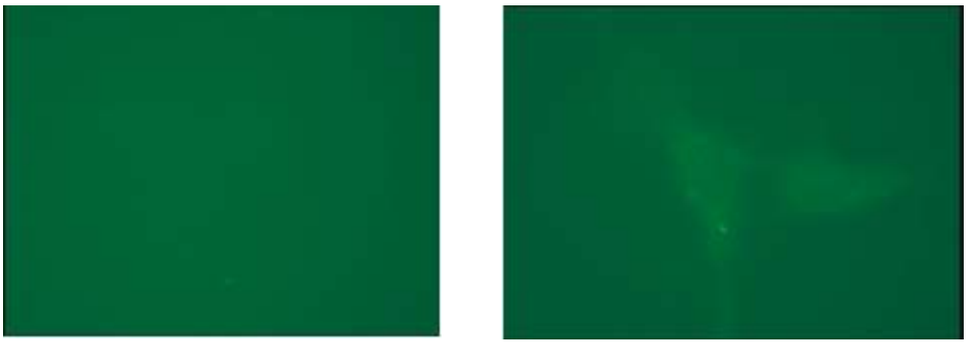
B Anukumar and AC Mishra

It was observed in our earlier study that expression of N gene in Vero cells induced cell lysis. The cells were also positive for TUNEL when employed TUNEL assay. Analyzing the expression of caspase-3 is one of the methods to confirm the involvement of apoptosis.

Work done

N gene mammalian expression

N gene transfected Vero cell was stained with anti caspase-3 antibody conjugated with FITC. It was observed that these cells are positive for caspase-3. This experiment indicated that expression of N gene in Vero cells induced apoptosis through caspase-3 pathway.



Mock transfected cells (100X) N gene transfected cells (100X)

Fig.1: Staining of caspase-3 in N gene transfected Vero E6 cells

Expression and purification of glycoprotein (G) of Chandipura virus in prokaryotic expression system

Full length G gene was cloned into pET 32b vector. The positive clone was transformed into BL-21 host. The protein was expressed as inclusion body. The inclusion body was isolated and the G protein was purified. The purified protein was refolded using sucrose. The refolded protein was further purified by ion exchange chromatography. Purity was checked by SDS-PAGE and western blot.

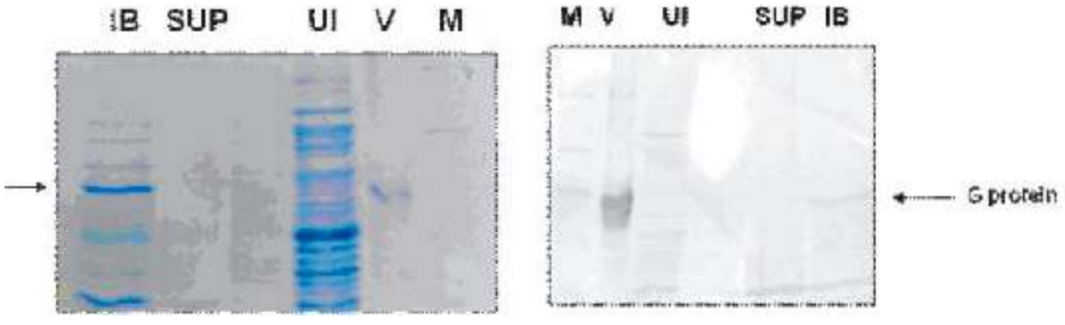


Fig.2: G protein expression in *E.coli* system. IB-Inclusion body, SUP-supernatant, UI- un induced, V- virus, M- marker

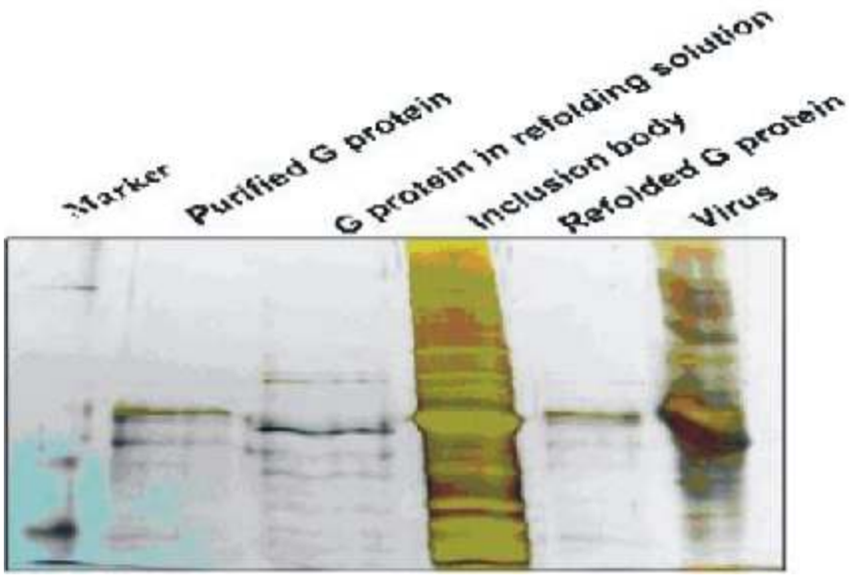


Fig.3: Refolding of expressed G protein

Chandipura virus encephalitis: The role of virus interaction with toll like receptor 4

B Anukumar and AC Mishra

The cytokines expression is negatively regulated by different SOCS proteins using different pathways. Some viruses exploit this mechanism for their replication by inhibiting Type I interferons signaling. In this study the phenomenon was checked in RAW cells.

Work done

(a) Role of suppressor of cytokine signaling (SOCS) protein in CHP pathogenesis

CHP infection of RAW cells induced the expression SOCS1, 2, 3 and CIS protein. This cell also expressed IFNα/β and IFNα/β receptor. The induction level was quantitated in transcript level by semi quantitative RT-PCR. These SOCSs proteins might suppress the Type I IFN activity **(Fig.4, 5)**

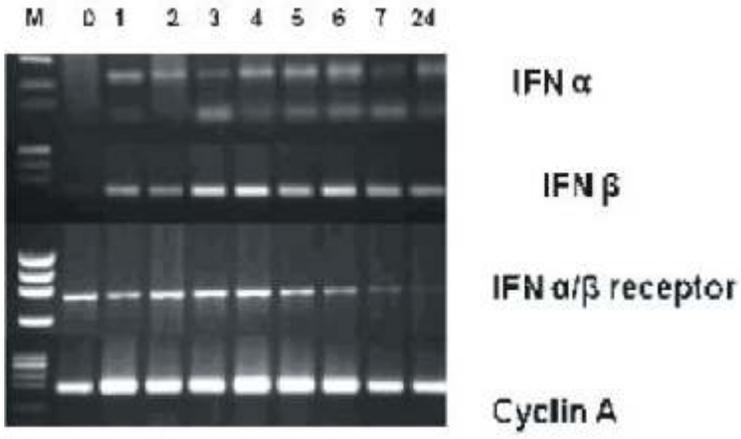


Fig.4: Transcript level of Interferon a/í and Interferon a/í receptor expression in Chandipura virus infected RAW cells at different hours post infection

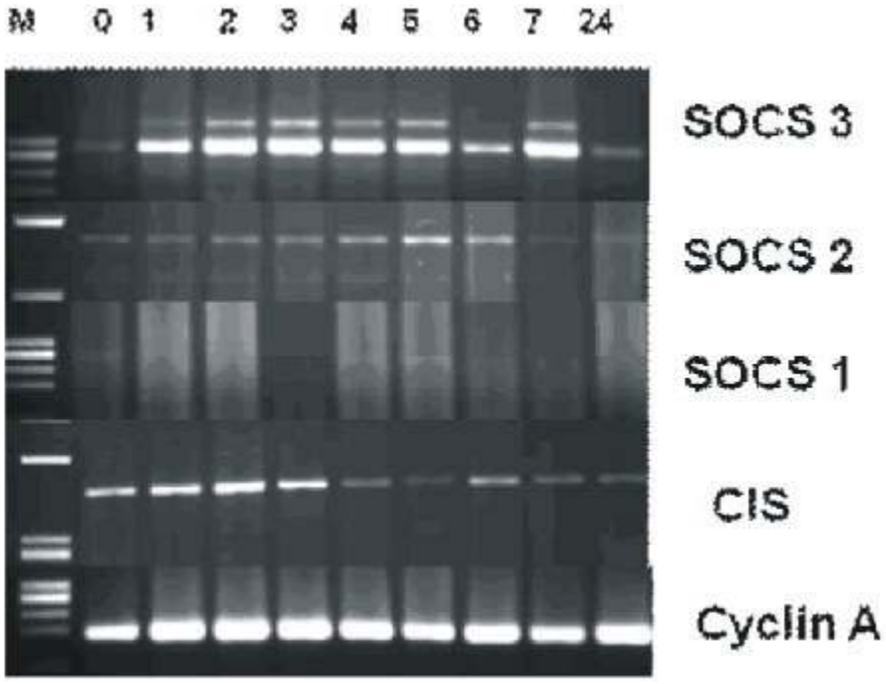


Fig. 5 : Transcript level of SOCS and CIS expression in Chandipura virus infected RAW cells at different hours post infection

(b) Role of TNFa in CHP pathogenesis

In vitro it was observed that compare to the unprimed RAW cells the TNFa primed RAW cells synthesis more virus particles. Similarly inhibition of TNFa either by small molecule TNFa inhibitor or neutralizing antibody reduced the virus replication. This indicates that TNFa, suppress the host cell defense mechanism through SOCS pathway **(Fig.6)**.

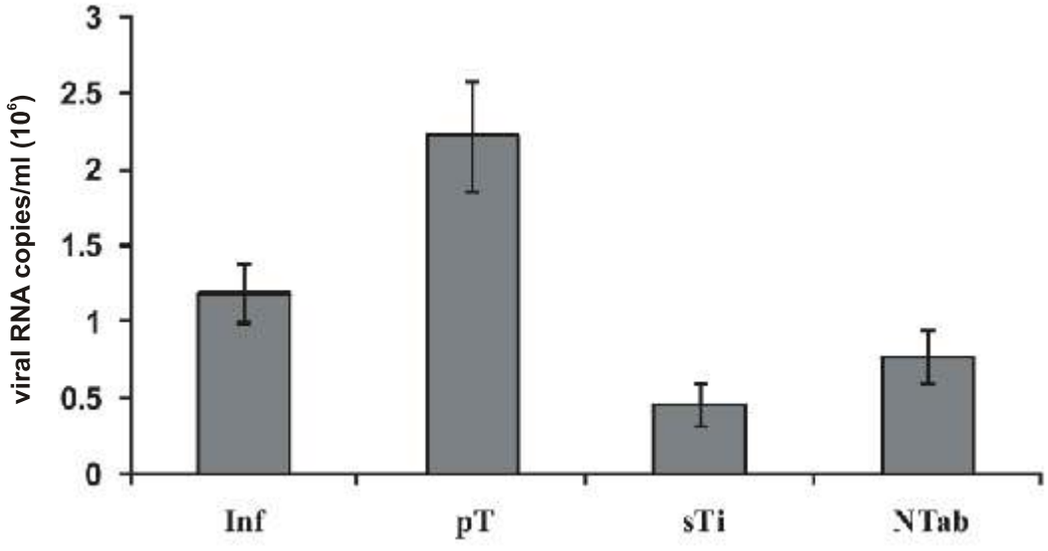


Fig.6 : Virus titer in supernatant of RAW cells infected with virus by real time RT-PCR.

Inf: infected cells, **pT**: TNFα primed cells, **sTi**: small molecule TNFα inhibitor treated cells

NTab: TNFα neutralizing antibody treated cells.

Most important finding

How the Chandipura Virus overcomes the protective innate mechanism of cell was ruled out in this study. It was observed that Chandipura virus stimulate the TLR4 receptor in RAW cells. Stimulation of TLR4 leads to the secretion of proinflammatory cytokines especially the higher level of TNFa. Sensitization of these cytokines by cells inherently induce the synthesis of suppressor of cytokine signaling protein to prevent the damage of host cells by excess secretion of proinflammatory cytokines. These proteins bind to the cytokine receptor to prevent the downward signaling. These signaling affect invariably most of the cytokine signaling including Type I interferons, which protect the cell from virus infection. So the cells lose the protective role and allow the virus to replicate inside the cells. This is the overall mechanism by which Chandipura virus infects the cells and replicate inside the cells.

Cloning and expression of G-gene of Chandipura virus and evaluation of immunogenicity in mice

Venakateswarlu.Ch, VA Arankalle

Introduction

Encephalitis caused by Chandipura virus (CHPV) has emerged as an important pediatric health problem in India as evidenced by the epidemics of the disease with high mortality in 2003 in the state of Andhra Pradesh (183/329, 55%), in 2004 in the state of Gujrat (20/26, 78.4%) and in 2007 in Maharashtra and Andhra Pradesh states (our unpublished observations). These fatalities occur within 48 hours of appearance of clinical symptoms, majority being within 24 hours. No specific treatment or vaccine is available. Since the problem is geographically focussed at this time, an effective vaccine is desirable for this dreadful disease.

Objectives

- To develop recombinant G-protein-based candidate vaccine for Chandipura infection.

Work done

Expression of G-protein (rGp) and its evaluation as an antigen in the development of IgM/IgG-anti-CHP ELISA was reported last year. This year evaluation of rGp candidate vaccine was pursued.

Purification of rGp

Fig. 7 depicts elution profile of the serum-free concentrate of the rGp positive SF9 cell culture supernatant loaded on gel filtration HPLC column. All the fractions were subjected to ELISA for rGp detection. Among these, only fractions corresponding to a single peak (Peak: 13.61) were scored as reactive. Similar to culture supernatant, this rGp peak was confirmed by SDS-PAGE and Immunoblotting to be a single protein of approximately 60-kDa (expected size of rGp). This protein was used for mice immunization.

Humoral Immunity

Both pre and post-immunization mice sera were subjected to ELISA and NT for the detection of anti-CHPV antibodies. Both assays detected antibodies as early as two weeks after 1st dose **(Table-1)**. Pre-immunization sera were scored negative by both the tests. As compared to NT, ELISA detected seroconversion earlier when the dose

of the immunogen was low (100 ng). Antibody response was immunogen concentration dependent, increasing gradually with dose from 100ng-1µg. Both 1 and 2 µg gave comparable response. Percentage sero-conversion increased after each dose and the maximum (90%) sero-conversion was observed at 10th week of post immunization i.e. 2nd week of the last dose, either 1 or 2 µg.

Fig. 8 compares anti-CHP-antibody titers by ELISA and NT at 2nd week after the last dose. Similar to seroconversion rates, the antibody titers were function of the concentration of the immunogen used, 1 and 2-µg rGp producing comparable titers. The maximum titers in NT and ELISA were 1:320 and 1:1200 respectively. Neutralizing antibody titers were consistently lower than the ELISA titers. As evident from figure 4, irrespective of the dose of rGp used for immunization, the anti-CHP-antibody titers remained almost constant during the observation period of six months.

To assess the efficacy of the antibodies generated by immunizing with the 2003 isolate-derived rGp against the viruses isolated in 1965, 2004 and 2007 neutralization tests were performed employing different virus isolates **(Table 2)**. Serum samples from five immunized mice were screened in NT with the homologus as well as heterologus CHPV isolates. An excellent cross protection was recorded; NT titers employing different isolates did not differ significantly (p=0.423 to 0.510).

Cell Mediated Immunity

To assess the T cell-response, spleens from 10 mice from each group harvested at 2-3 week after the last immunization were used. The optimum concentration of rGp for the stimulation of spleen cell was found to be 20µg/ml. Table 3 records stimulation indices for the mice belonging to different groups. Similar to humoral immune response, lymphocyte proliferative response increased in terms of stimulation index, with the dose of the immunogen. Sixty percent of mice immunized with 1 or 2 ug rGp responded with high SI values.

Intra-cerebral virus challenge experiments:

An intracerebral challenge of 100LD_{50} of the homologus strain yielded satisfactory results. Survival was directly proportional to the immunogen dose, 20 and 40% mice surviving with 100 and 500ng doses respectively. Both 1 and 2 ug doses gave 90% protection. Mortality in control, un immunized group was 100%. As evident from the table 4, an ELISA titer of 1:40 and NT titer of 1:20 was predicting protection against the intracerebral challenge. Overall, 1ug purified recombinant G protein expressed employing Baculovirus Expression System was shown to be a promising candidate vaccine.

Table-1 Percent seroconversion in mice immunized with three doses of different concentrations of rGp at 0th, 4th, 8th and 10th weeks as documented by ELISA and NT.

	% Sero conversion at 0 th week I 4th week I 8th week I 10th week							
	ELISA	NT	ELISA	NT	ELISA	NT	ELISA	NT
100 ng	0	0	20	10	50	40	40	40
500 ng	0	0	30	20	50	50	60	50
1 ug	0	0	40	40	70	70	90	90
2 ug	0	0	40	40	80	80	90	90

Table 2: NT titers of serum samples from immunized mice employing homologous and heterologus CHPV isolates.

Mice No.	NT Titer of CHPV isolate 034627	NT Titer of CHPV isolate 653514	NT Titer of CHPV isolate 2004	NT Titer of CHPV isolate 076324
1	80	40	40	20
2	320	160	160	320
3	20	40	20	40
4	160	80	80	160
5	320	160	160	80

Table 3: T cell proliferation in response to rGp as measured by stimulation Indices in different mice groups

Mice group (n=10)	S.I. value range (Mean ± Std error)	No of responder mice (Percentage)
100ng	0.95 to 8.57 (2.17 ± 0.68)	2 (20)
500ng	1.09 to 11.45 (3.50 ± 1.02)	3 (30)
1ug	1.15 to 15.82 (7.73 ± 1.69)	6 (60)
2ug	1.21 to 16.26 (8.35 ± 1.78)	6 (60)
$AlPO_4$	1.26 to .2.56 (2.21 ± 0.07)	0

Table 4: Comparison of survival following intracerebral challenge of CHPV with antibody titers as estimated by ELISA and NT

rGp Conc.	No. of mice showing ELISA titers of 1:40, (n=10)	No. of mice showing NT titers of 1:20, (n=10)	Survival status (100 LD_{50}), (n=10)
100ng	3	2	2
500ng	5	4	4
1µg	9	9	9
2µg	9	9	9
$AlPO_4$ alone	0	0	0

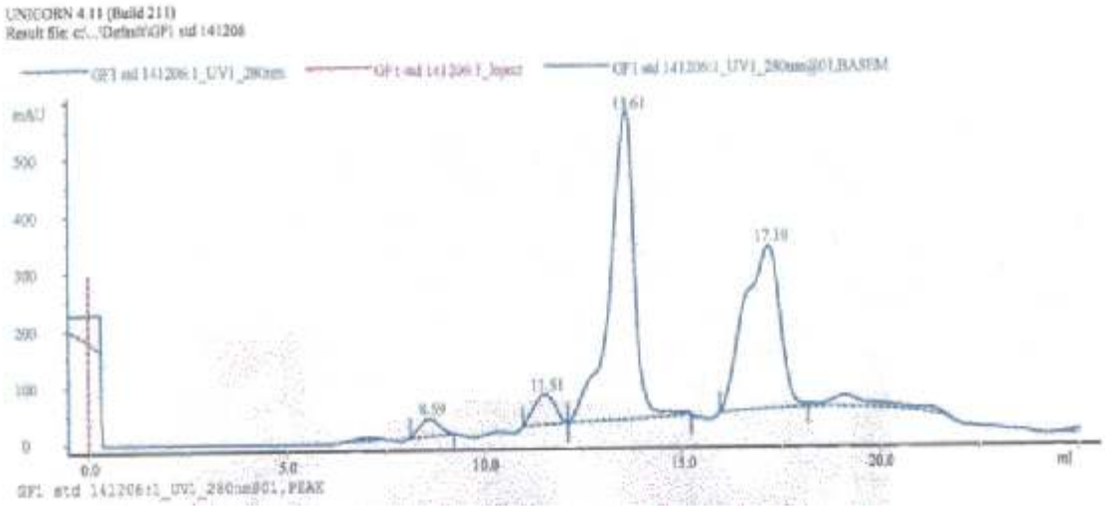


Fig. 7: Elution profile of the serum-free concentrate of rGp positive SF9 culture supernatant loaded on gel filtration HPLC column. Peak: 13.61 corresponded to rGp.

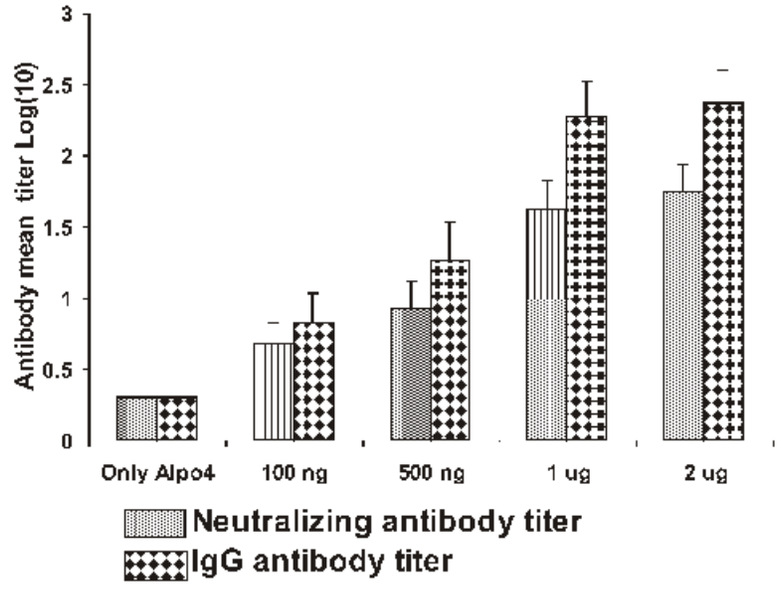


Fig. 8 : The Geometric mean of reciprocal anti-CHPV antibody titers (Log10) at 10 weeks i.e., 2 weeks after the third dose of rGp. Bar represents standard error.

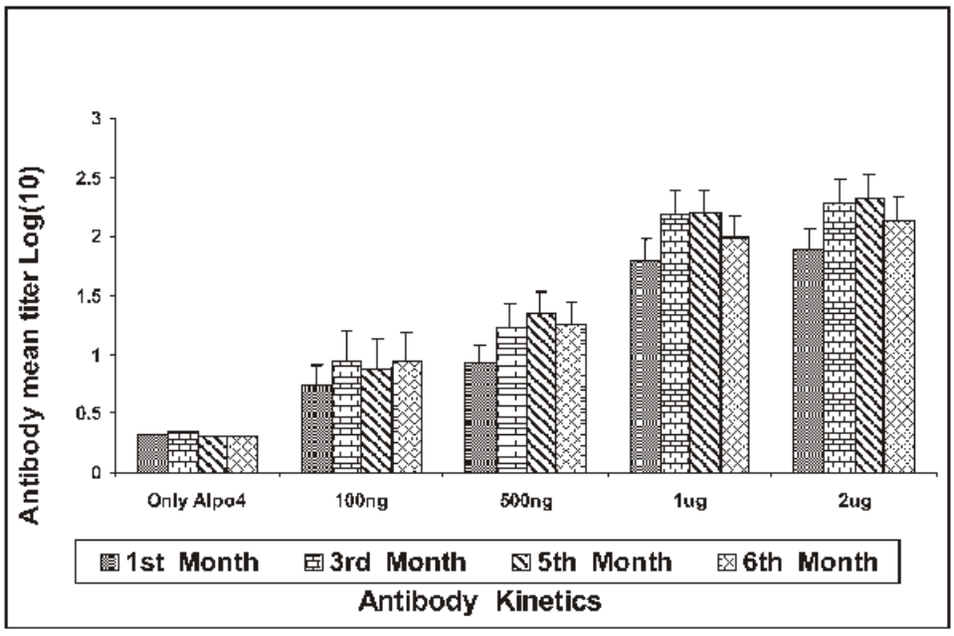


Fig. 9 : The Geometric mean of reciprocal IgG-anti-CHPV antibody titers (Log10) as determined by ELISA. Antibody titers were determined 1, 3, 5 and 6 months after the third dose of rGp. Bar represents standard error.

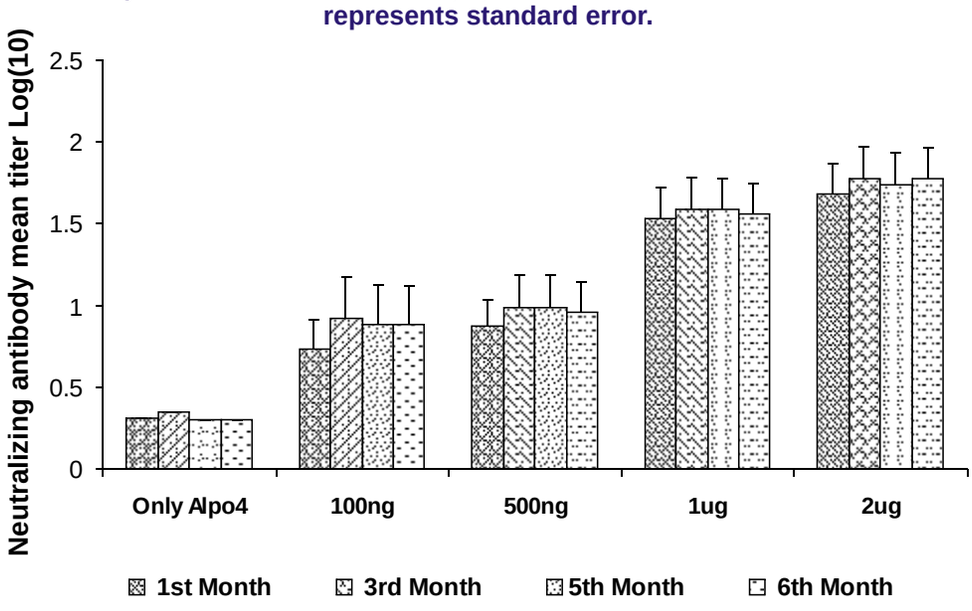


Fig. 10 : The Geometric mean of reciprocal anti-CHPV neutralizing antibody titers (Log10) of as determined by in-vitro Neutralization test. Antibody titers were determined 1, 3, 5 and 6 months after the third rGp immunization. Bar represents standard error.

Evaluation of siRNA in curing Chandipura virus infection *in-vitro* and *in-vivo*

Satyendra Kumar, VA Arankalle

Introduction

Chandipura virus (CHPV) has emerged as an important encephalitis-causing pathogen in India. Outbreaks of the disease have been reported among children from the states of Andhra Pradesh, Gujarat and Maharashtra with mortality varying from 55-75%. There is no vaccine as well as therapy against CHPV infection. It was considered worthwhile evaluating the siRNA- based strategies to control the virus replication.

Objectives

- To assess the role of siRNA in controlling / inhibiting CHP virus infection.

Work done

P gene of Chandipura Virus isolate 034627 was amplified by RT-PCR by using PFloF1 and GFPPR1 primers and then both the vector and the insert were digested with the KpnI and SacI and ligated. After transformation colonies were screened and positive colonies were further sequenced to confirm the presence of P gene. To confirm the fusion protein western blot analysis was also done **(Fig. 11).**

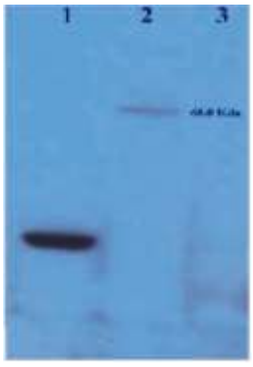


Fig. 11 : Western blot of P gene using GFP antibodies

P gene siRNA validation studies in 293 cells

Cells were transfected with both P gene siRNA and pAcGFP1N1-CHPV-P gene, which codes P protein with GFP as a fusion protein. Twenty-four hours after transfection cells were harvested and FACS and real time PCR was done. This data shows that out of four siRNAs (P1 to P4), P-2 is the best.

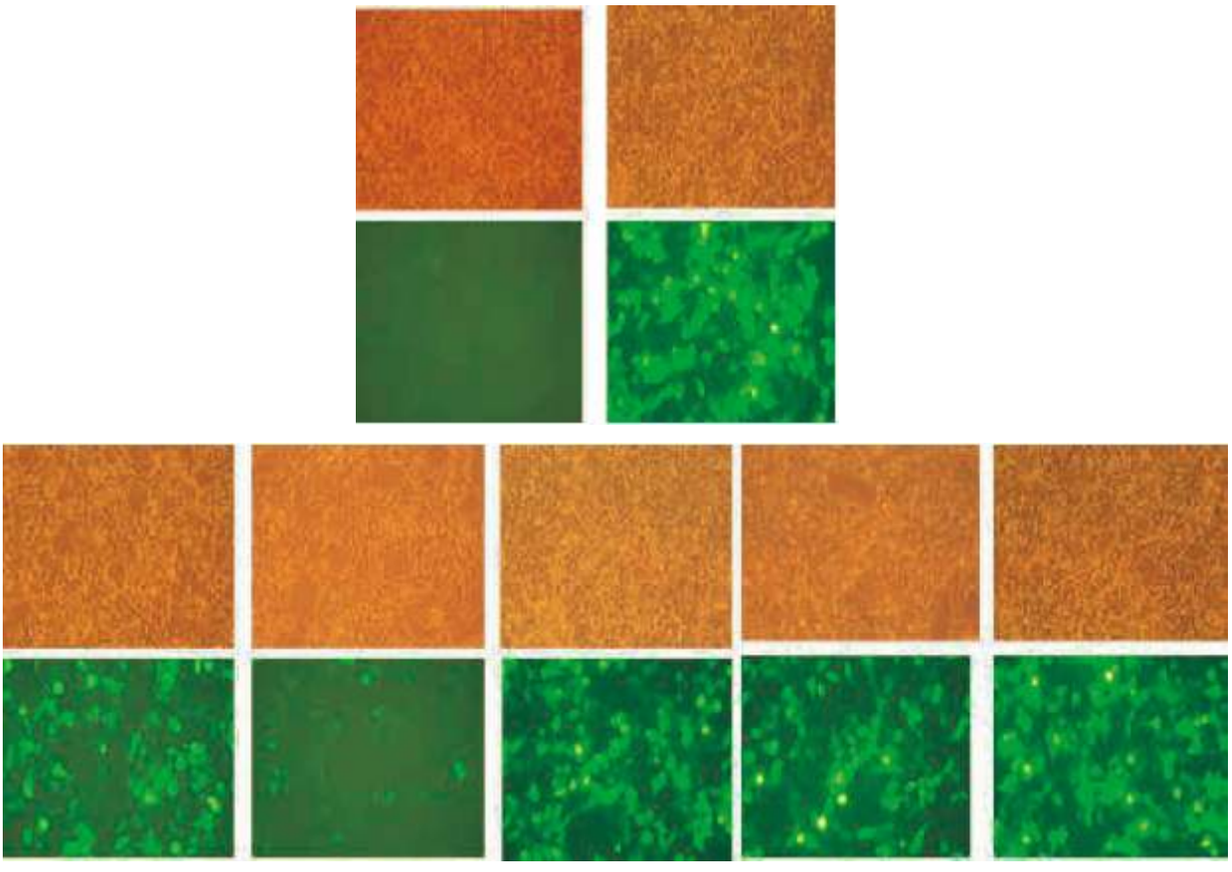
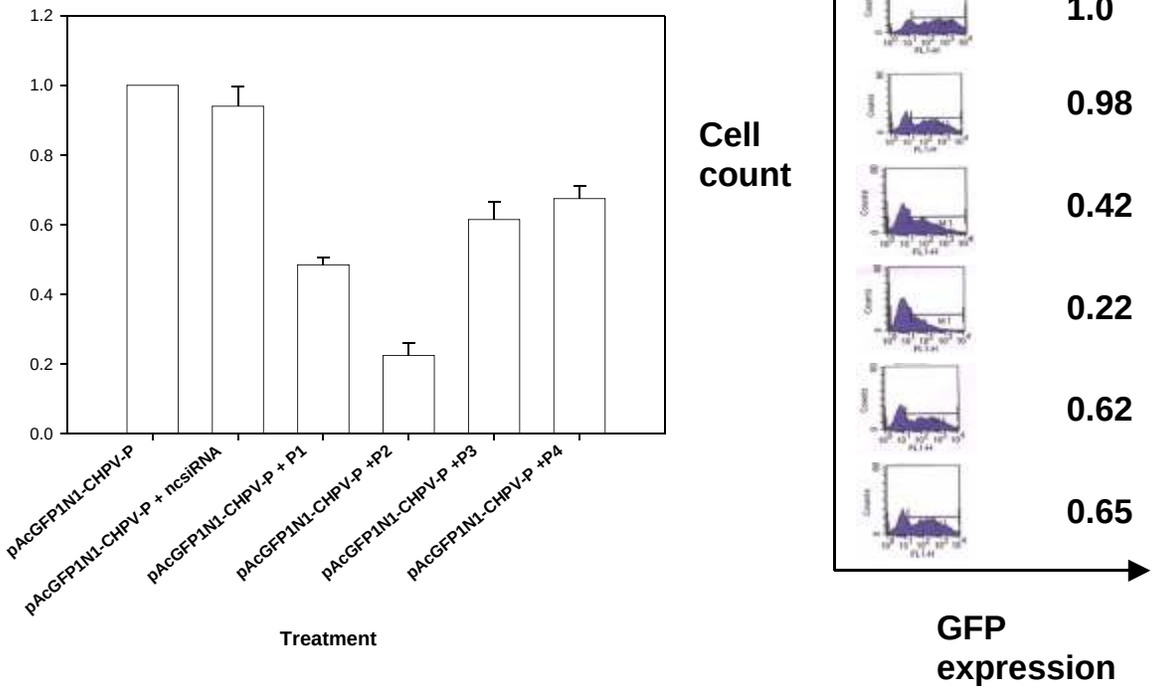
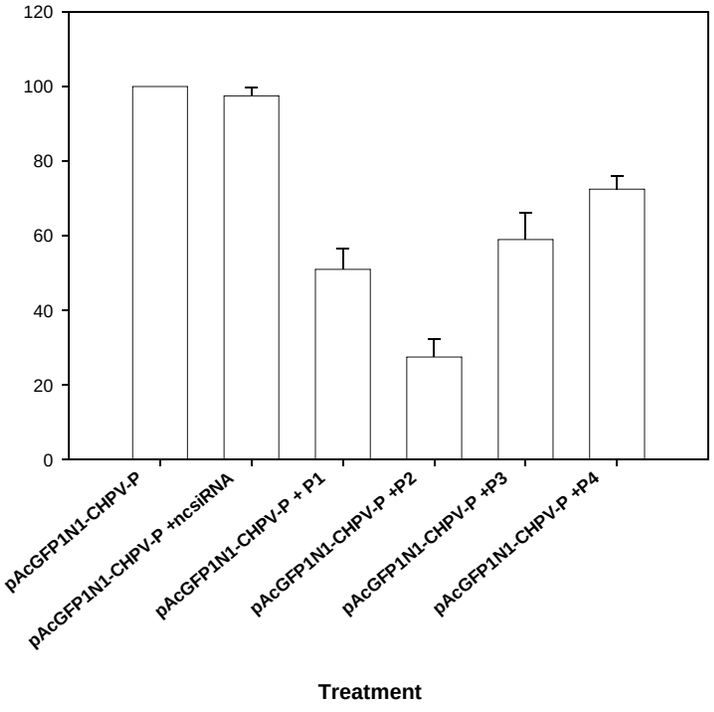


Fig. 12: Fluorescent microscopy of cells transfected with pAcGFP1N1-CHPV-P plasmid and different siRNA (P1-P4)



(a) (b)



(c)

Fig. 13 (a) (b) and (c) P gene siRNA Validation by flow cytometry and real time one step RT-PCR.

Optimization of concentration of P-2

Different concentrations of P-2 siRNA were used in combination with pAcGFP1N 1-CHPV-P gene FACS and Real time analyses were done.

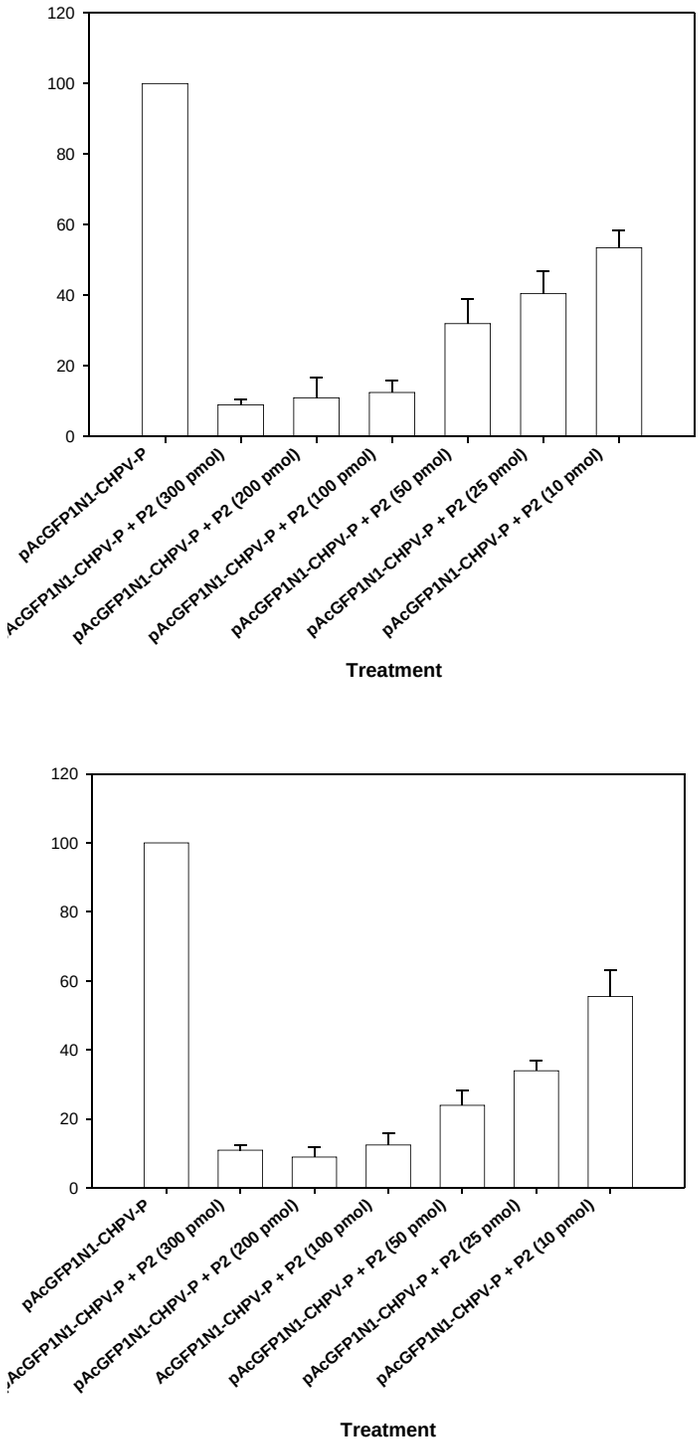
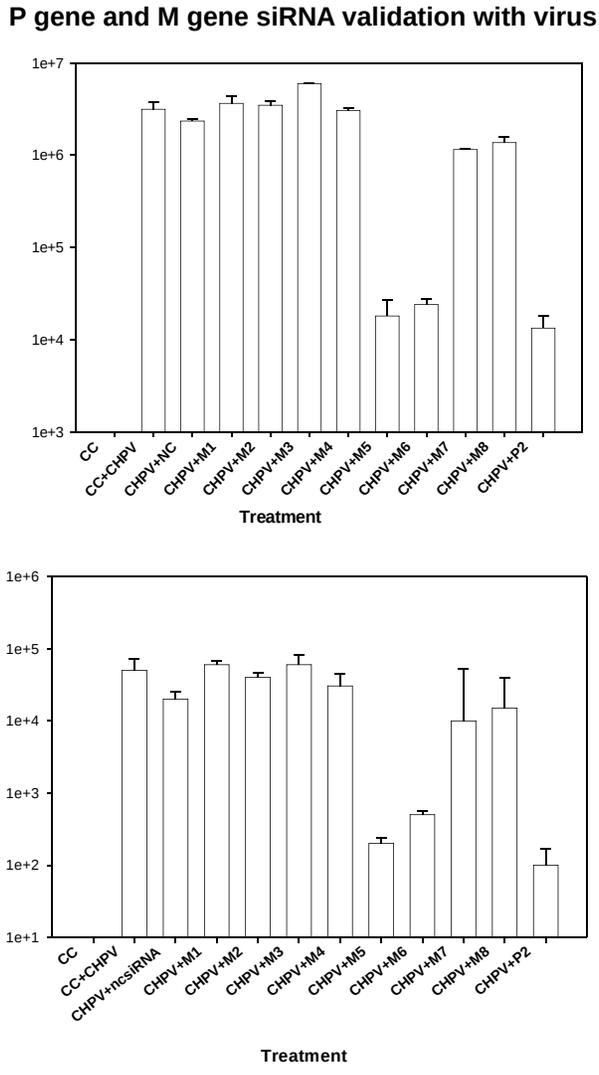
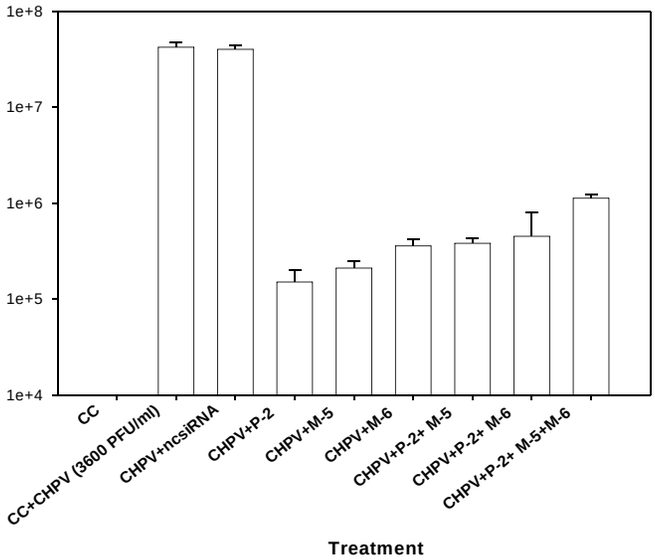


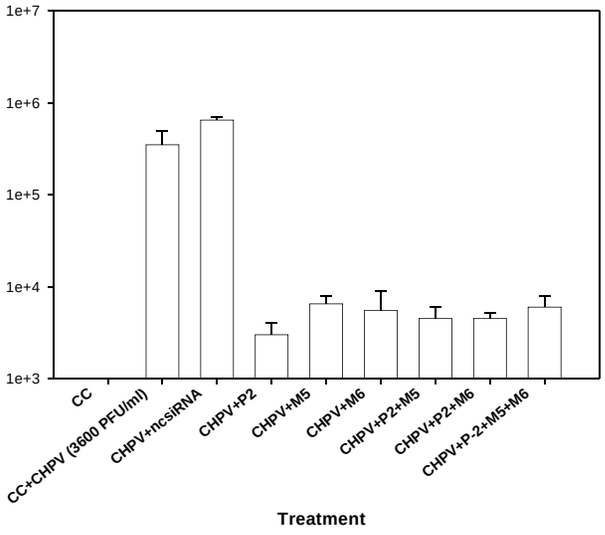
Fig. 14 : Optimization of concentration of P-2 by flow cytometry and real time one step RT-PCR



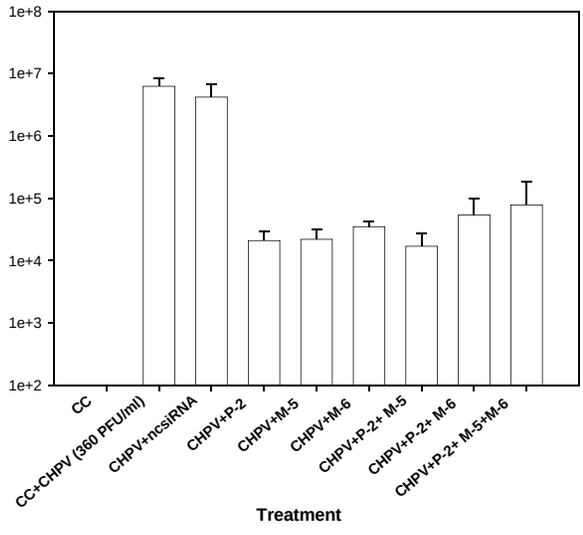
P gene siRNA, P2 and all the M gene siRNA were used for the transfection and 24 hour after transfection cells were infected with 360 pfu of CHPV. 24-hour PI cells were harvested and real time was done.



(a)

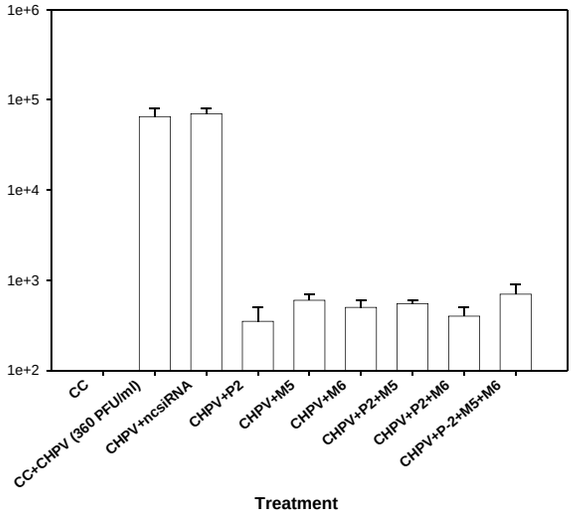


(b)

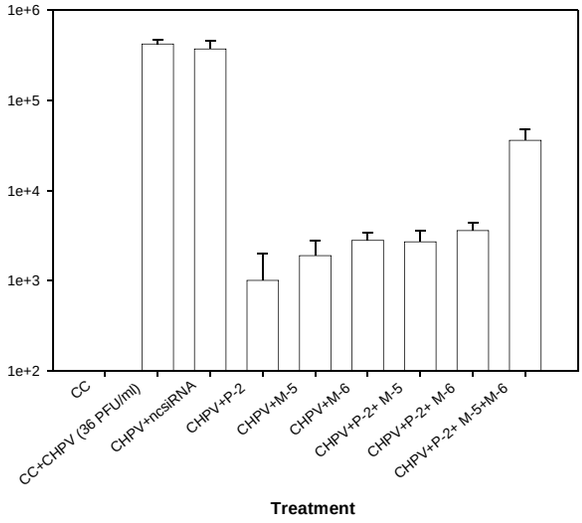


(c)

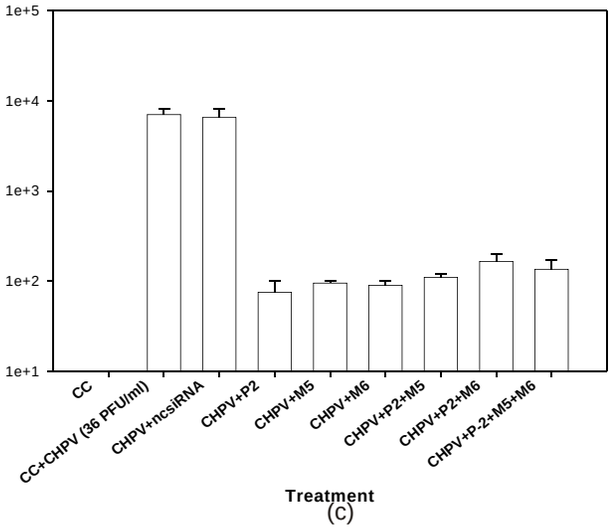
Fig. 15 : Different M gene and P-2 siRNA validation using CHPV



(a)



(b)



(c)

Fig. 16 : Effect of different virus concentration and different combination of siRNA on the replication of CHPV (a), (b) and (c)

Conclusion

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HLA Phenotypes and outcomes of Chandipura virus infection in Andhra Pradesh

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Introduction

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Work done

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Future Plan

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In-silico sequence and structure-based antigenic analysis of the G, N and M proteins of Chandipura virus

ßJe*E`P…ºdšÏÍÜ| ¼CyÏ:uªÿS

The Chandipura virus (CHPV) is emerging as a fatal infectious disease with very high mortality rate. Hence it would be apt to use in-silico sequence and structure based approaches to determine epitope driven vaccine candidates. Among (CHPV) structural proteins, glycoprotein G, the spike protein protrudes externally from the outer membrane of the virus particle and elicits antibody response thus acting as a major antigenic determinant (Neumann et al. 2002). Matrix protein M lies in the inner surface of the virion to tether the nucleocapsid to the membrane. L and P are packaged within the mature virion and remain associated with the core nucleocapsid particle.

Whole genome sequence data is available for CHPV isolates from febrile as well as encephalitis cases. As far as 3D structures are concerned, the homologous crystal structures available are that of the pre and post fusion forms of the Vesicular Stomatitis Virus glycoprotein G, matrix protein of the Vesicular Stomatitis Virus (VSV) and a recent crystal structure of the Rabies virus N protein complexed with RNA. The G and N proteins are known to have immunogenic characteristics. Hence the sequential B - cell epitopes as well as the T - cell epitopes on these proteins can be predicted. Peptides containing these antigenic regions may help in the design of subunit vaccines.

Objectives

- Modeling of the G, N and M proteins
- Prediction of T-cell and B-cell (linear and conformational) epitopes

Results

The complete genome sequences of CHPV of five isolates have been determined from febrile and encephalitis outbreak cases during the period from 1965 to 2007. The genome length of approximately 11,100 nt shows closest sequence homology with the genomes of VSV and Isfahan virus (ISFV). Pairwise comparison of the deduced amino acid sequences of the 5 isolates of CHPV showed that the M protein is the most conserved (99.9% aa identity).

Sequence alignment of the G protein sequences of viruses of the rhabdoviridae family including the sequences of the CHPV isolates available has been carried out ensuring the alignment of conserved cysteine residues. Mapping of the known antigenic sites of VSV and Rabies virus indicates that the specific mutations of CHPV isolates fall in the antigenic site G3. Mapping of these site have been done on a tertiary structure model of the CHPV G protein. Homology modeling approaches with Modeler in Discovery studio, using the VSV 'G' protein as a template would be done . Scan Prosite was used to locate functional motifs in the CHPV sequences of the G, M, N, P and L proteins for characterization of the genome.

Pathogenesis of Chandipura virus in laboratory rodents (mice & rats)

CG Raut

Chandipura virus causes acute encephalitis to the humans especially children. Because of the acute nature of the disease not much information is available on pathogenesis. Hence attempts were made to develop suitable animal model for the disease by mimicking the natural route of infection.

Objectives

- To find out the infectivity of CHPV in mice of 16 days old and distribution of virus in the body by subcutaneous route of infection.
- Behavioral and histological response of CHPV infected rats after subcutaneous route of inoculation.

Work done

Subcutaneous inoculation of CHPV was done in mice of age 16 days to mimic the natural infection. Every post-infection day animals were sacrificed humanely. There were no gross changes in all the organs. Initially sickness was transient. After 5th PID hindlimb weakness was noted and it was continued to 7-8 PID and further leads to the recovery. Histological changes were observed in gradation post infection day wise only in brain and spinal cord.

Brain Sections: Histopathological observations

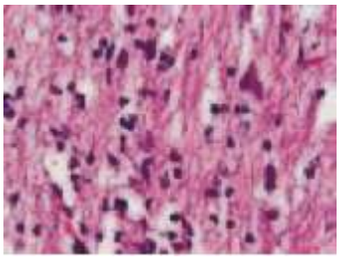

Fig.17 : 20X

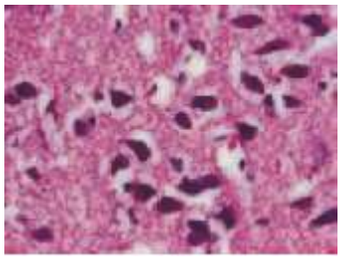

Fig. 18 : 60X

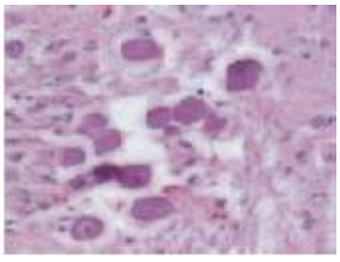

Fig. 19 : 40X

Fig.17 : Gliosis, degeneration of neurons
Fig. 18 : Shrinkage of neurons, vacuolation
Fig. 19 : Degeneration of neurons+++, Vacuolation, spongiosis.

Localization of antigen by immunohistochemistry in brain sections

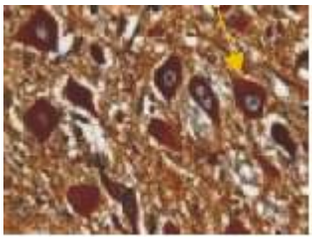

Fig. 20 : 40X

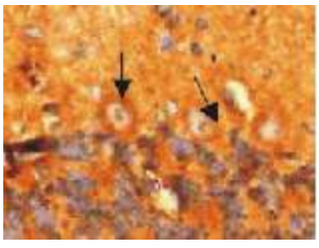

Fig. 21 : 60X

Fig. 22 : 20X

Fig.20: Antigen detection (dark brown color) in cytoplasm of neurons, chromatolysis of neurons.
Fig.21: Localization of antigen in Purkinje cells of neurons (dark brown).
Fig.22: Localization of antigen in choroid plexus.

Subcutaneous inoculation of CHPV was done in rats of ten days old to mimic the natural infection. Every post-infection day animals were sacrificed humanely. There were no gross changes in all the organs. Frank sickness was observed with ataxia, hyperasthesia, convulsions, quadriplegia and death. Marked histological changes were observed only in brain and spinal cord in gradation of post- infection period. Conclusively, rats of two weeks age could be suitable animals for studying the pathogenesis, host-virus interaction, drug development, etc for Chandipura virus.

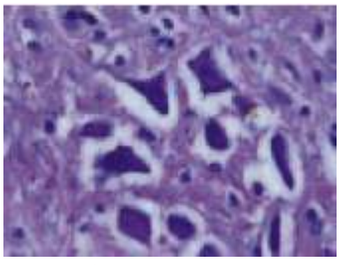

Fig. 23 : 40X

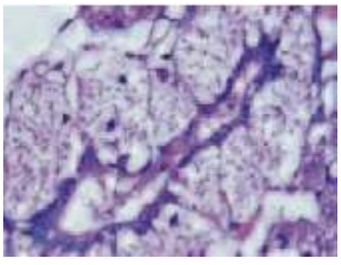

Fig. 24 : 60X

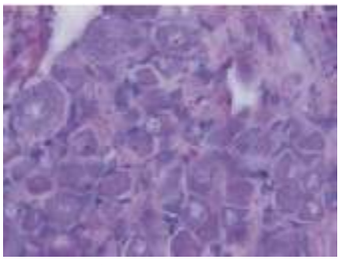

Fig. 25 : 20X

Fig. 23 Spinal cord : shrinkage of neurons leading to vacuolation
Fig. 24 Spinal cord : degeneration of nerve roots around the central canal
Fig. 25 Spinal cord : degenerative changes in the nerve roots.

Future plan

- To study in detail the progressive pathological changes in different parts of brain of the host (mice/rats/hamsters).
- Neuropharmacological studies of infected hosts to understand the changes in different neurohormones.
- Invivo imaging of infected animals to understand the progression of disease without sacrificing the animals.

Workshops / Conferences / Seminar / Meetings attended

- Anukumar, B and Mishra, AC, oral presentation entitled “Chandipura virus infected raw 264.7 cells regulates CD4+ and CD8+ levels by secretion of pro-inflammatory cytokines and expression of Fas ligand” in the, 34th Indian Immunological Society Conference, NARI, Pune December 16-18, 2007.
- Venkateshvaralu CH and VA Arankalle, oral presentation entitled 'Recombinant glycoprotein based vaccine for Chandipura virus' at '34th Indian Immunology Society Conference', at National AIDS Research Institute, Pune, during December 16-18, 2007.

Chikungunya

Chikungunya

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6 Chikungunya

- Detection and Quantification of Chikungunya Virus (CHIKV) RNA by real-time RT-PCR
- Evaluation of RNAi (RNA interference) technology in curing Chikungunya Virus infection in-vitro and in-vivo
- Study on pathogenesis of Chikungunya virus (CHIKV) in laboratory animals
- Host immune response/s in Chikungunya cases hospitalized with diverse clinical presentations
- Association of the neurological complications of the Chikungunya virus infection during the current epidemic with specific mutation(s) in the viral genome
- Establishment and characterization of a new *Aedes aegypti* cell line for Chikungunya isolation and propagation
- Growth kinetics of Chikungunya virus in certain cell lines

Detection and Quantification of Chikungunya Virus (CHIKV) RNA by real-time RT-PCR

Deepti Parashar, MD Gokhale, GP Jacob, S Kumar, AC Mishra and VA Arankalle

Introduction

Chikungunya virus (CHIKV), a member of the alphavirus genus, is of considerable public health concern in Southeast Asian and African countries. However, despite serological evidence, the diagnosis of this arthropod-borne human disease is confirmed infrequently and needs to be improved. In fact, illness caused by CHIKV can be confused with diseases such as dengue or yellow fever, based on the similarity of the symptoms, and laboratory confirmation of suspected cases is required to launch control measures during an epidemic.

Objectives

- To develop TaqMan real-time RT-PCR method for detection and quantitation CHIKV RNA
- Demonstration of its utility in documenting CHIKV TOT in field collected and experimentally infected *Aedes* mosquitoes

Work done

CHIKV Primers and probe designing: All the full genome sequences of CHIKV available in GenBank (or our unpublished sequences) were aligned and conserved region were targeted to locate the primers and TaqMan MGB probes using primerexpress software (Applied Biosystems Inc, Foster, CA).

Construction of CHIKV standard: A PCR product encompassing the targeted region was prepared using the confirmed CHIKV strain and cloned into the T7 polymerase expression vector pGEM-T-easy (Promega, Madison, USA) according to manufacturer's instructions. Clone with sense orientation was cut with Spe I and run off transcription was done with T7 polymerase. Transcript was purified, serially diluted and used as RNA standard. Viral RNA was extracted from samples using QIAamp viral RNA mini kit (Qiagen, USA).

The insert sequences for the CHIKV standard were confirmed by using big dye terminator kit on 310 sequence analyzer (Applied Biosystems).

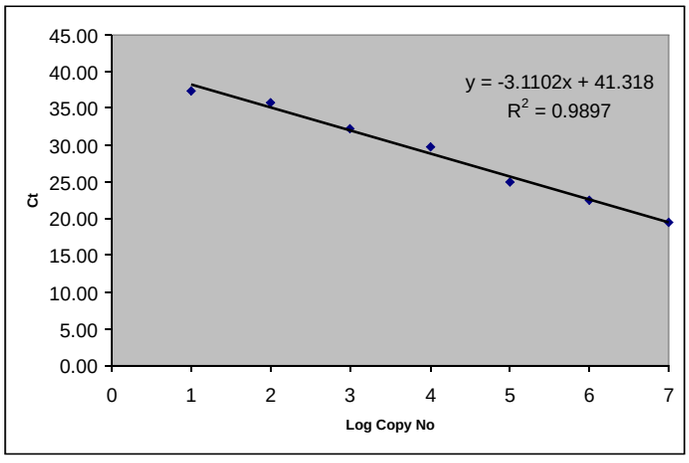


Fig. 1: Standard curve for the CHIKV-specific one step real time RT-PCR assay generated from the amplification plots between the 10-fold serially diluted IVT RNA. Serial 10-fold dilutions of *in vitro* transcribed RNA standard were plotted against the threshold cycle.

Real-time TaqMan RT-PCR assays. One-step real-time RT-PCR assay was performed using an ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA) as previously described.

Sensitivity, specificity and reproducibility of the TaqMan assay: The analytical sensitivity and reproducibility of the real time TaqMan RT- PCR was determined using a 10 fold dilutions of in vitro transcribed CHIKV strain in duplicate.

Specificity of CHIKV assay was evaluated by using infected cell extracts with DEN, JE and WN Viruses.

The detection limit of the assay with the CHIK standard template was 10 copies.

No cross reactivity was detected with DEN, JE and WN viruses.

Detection of CHIKV in clinical samples and in *Aedes aegypti* mosquitoes by real time RT-PCR method:

Optimization of RNA isolation procedure: Experimentally infected mosquitoes for detection of CHIKV in a pool of single mosquito as well as one positive mosquito in a pool of 50 were used.

TaqMan real-time RT-PCR method was developed and applied for detection and quantitation of CHIKV RNA in 198 clinical samples collected from Kerala, 36 samples were detected by this system. Out of 198 field collected and pooled mosquitoes (1-50 mosquito(es) / pool), 27 were found to be positive for CHIKV RNA. All the *Aedes aegypti* larvae collected from Andhra Pradesh were found to be negative for CHIKV RNA.

Evaluation of RNAi (RNA interference) technology in curing Chikungunya Virus infection *in-vitro* and *in-vivo*

Deepti Parashar, S Kumar, AB Sudeep, AC Mishra and VA Arankalle

Introduction

Chikungunya is an emerging arboviral infection of immense public health concern in Southeast Asian and African countries. Recently several parts of Indian Ocean islands and India witnessed explosive, unprecedented epidemic. The response to available treatment modalities is not impressive. The advent of RNA Interference--a phenomenon of sequence-specific degradation of RNAs mediated by double-stranded RNA--holds promise as a potential therapy for CHIKV infection.

Objectives

- To evaluate the efficacy of siRNA in inhibiting CHIKV replication in mosquito cells.
- To evaluate the efficacy of siRNA in inhibiting CHIKV replication in animal model.

Work done

siRNA oligos for E 2 gene were used for transfection and 24 hr after transfection cells were infected with CHKV. Cells were observed every 24, 36 and 48-hour PI cells were harvested and real time PCR was done.

Study on pathogenesis of Chikungunya virus (CHIKV) in laboratory animals

DR Patil and VA Arankalle

Introduction

India experienced re-emergence of Chikungunya (CHIK) virus after a quiescence of more than three decades in the form of explosive outbreaks affecting several states and 1.3 million people which may be a gross underestimate This re-emergence was associated with a shift in the genotype of the virus from Asian to African. Clinical complications and deaths were noted during the outbreak, especially in elderly people. Joint pain persisting for months together caused disability and heavy economic losses. Very rapid spread of the virus and increased virulence warrants detailed studies of the virus as well as the disease pathogenesis.

A systematic study in animals yields valuable information and paves way for further in-depth research. Considering this, the study was undertaken with following objectives.

Objectives

- To study tissue tropism and persistence of virus with special reference to joint and associated tissues
- To characterize tissue injury at macro and micro level.
- To understand involvement of different immune cell types in inflammation.

Work Done

Virus stock preparation

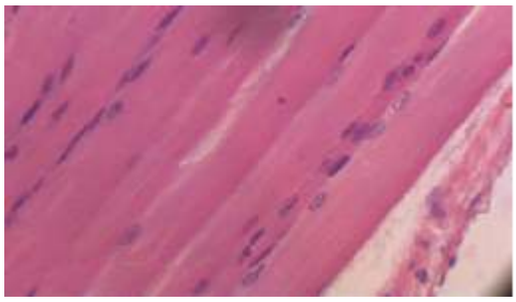
Latest Indian strain of CHIKV (061543): A serum isolate obtained from patient from Andhra Pradesh in 2006 was taken as a representative of 2006 outbreaks. Virus at mouse brain passage level 12 was tested for the susceptibility in mice at different ages by S/C route. As no overt clinical signs ere noted, it was further passaged six times in suckling mice by S/C route. Virus at MB12 level was inoculated by S/C route and the whole carcass was harvested except viscera and skin. It was homogenized and suspension was prepared. This carcass suspension was used to inoculate at next passage. Likewise six passages were made (MC1 to MC6) by S/C route in left inguinal region of suckling mice. Virus stock was prepared in Vero E6 cells using CHIKV at passage level MB12+MC6. Thus the stock virus used for the study was at passage level: MB12+MC6+V-1.

Susceptibility of 8-day-old Swiss Albino and C57BL/6 mice to MB12+MC6+V-1 virus: Swiss Albino and C57BL/6 mice of 8 day old age were inoculated with $10^{4.6}TCID_{50}$ of MB12+MC6+V-1 in the left inguinal region and mice were scored for development of hind limb disfunction and disease Hind limbs stiffness started at 6^{th}PID, which became severe on 8^{th} PID. Hind limb weakness could be observed on 12^{th} PID also though less severe as compared to 8^{th} PID. Necropsy revealed white patches on the hind limb thigh region.

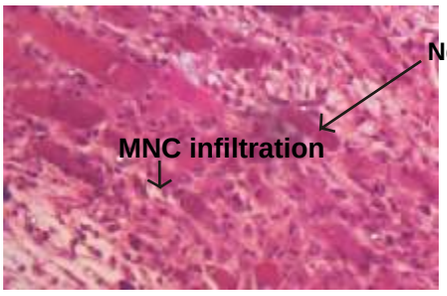
Characterization of tissue injury in 8-day-old Swiss Albino and C57BL/6 mice by histopathology:

Swiss Albino and C57BL/6 mice of 8-day-old age were inoculated with $10^{4.6}$ $TCID_{50}$ of MB12+MC6+V-1 virus by S/C route in the left inguinal region. Different tissues were collected from mock-inoculated control and infected age mached mice at 1, 3, 8 and 12 days post infection (DPI) and fixed in 10% formal saline Tissues were processed for histopathology. Paraffin sections of 5 µ were prepared, stained with Hematoxylin & eosin and evaluated for the microscopic changes

Muscles and joint from ipsilateral as well as contralateral (with respect to injection site) hind limbs of the mock-inoculated control animals were apparently normal without any lesions. Muscles of the hind limbs (ipsilateral as well as contralateral) of the 8 and 12-day post infection animals revealed severe inflammatory lesions such as mononuclear cell infiltration, oedema, moderate to severe muscular degeneration and necrosis with proliferation of fibrous connective tissue. Muscles of left limb (injection site) from 3-day post infection showed mild inflammatory lesion. Joint sections of mouse at 8-day post infection revealed minimal inflammatory cell infiltration (mononuclear) in joint space. Clinical symptoms in the form of hind limb stiffness correlated well with pathological changes observed in hind limbs. Histopathology examination of the liver, spleen, heart, kidney, and brain did not reveal any infection related lesions.



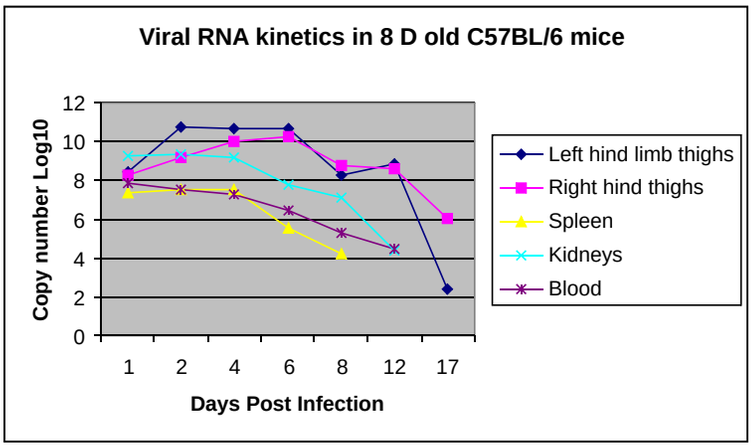
H&E, 40x- 12th PID Control



H&E, 40x- 12th PID infected

Viral RNA kinetics by real time RT PCR in infected 8 day old C57BL/6 mice: Different tissues and whole blood were collected at 1, 2, 4, 6, 8, 12 and 17days post infection (DPI) and stored in RNA letter. At each time point three animals were sacrificed and the tissues and whole blood were collected in RNA letter separately. For each set of tissues one mock-inoculated animal was sacrificed and the tissues and blood were collected in RNA letter similar to infected animals. Tissues and blood were stored at 70°C till assayed.

Viral RNA load was found maximum in the hind limbs (Ipsilateral as well as contralateral). In limbs, viral RNA remained at peak from 2nd to 6th PID (10 11 logs per gram of tissue). Thereafter, declining at 8th PID but still maintaining heavy load through out the observation period (8-9 logs per gram of tissue at 12th PID). Contrary to which, the viral RNA number declined faster in spleen through out the observation period (4 logs per gram of tissue on 8th PID). Decline was comparatively slower in blood (4-5 logs per ML of blood on 12th PID). Ironically the viral load in kidneys was found much higher (8-10 logs per gram of tissue from 2nd to 4th PID). Clearance from kidneys was also slower as compared to spleen and blood Gross and histopathological lesions in the limbs were seen maximum on 8th PID, the time point at which, decline in viral RNA load was observed in most of the limb tissues assayed.



Tissue : Copy number is measured per gram of tissue.

Blood : Copy number is measured as per ml of blood.

Each data point represents arithmetic mean of three mice.

Host immune response/s in Chikungunya cases hospitalized with diverse clinical presentations

Anuradha Tripathy, BV Tandale, SN Ranadhive and VA Arankalle

Introduction

Chikungunya (CHIK) has re-emerged in an explosive epidemic form in Indian Ocean islands and India. An estimated 1.38 million people from 11 states in India were severely affected by Chikungunya virus (CHIKV) infection. During the current epidemic, it was observed that Ahmedabad, the capital of the state of Gujarat experienced clinical complications of CHIKV infection leading to hospitalizations. These included encephalitis and multi-systemic involvements and classical fever cases. A possible role of underlying medical conditions in the progression of the CHIKV infected individuals to a variety of complications was envisaged. Atypical clinical presentations could be attributed to the host factors and/or critical mutations in the viral genome. Such studies have not been reported so far for CHIKV. In fact, even in classical CHIK infection, the dynamics and role of both humoral as well as T cell immune responses have not been established, probably because of the rarity of the disease. No data is available on cellular immune responses as well. Cellular immune responses play important role in the protection against viruses.

Objective

- To understand the cellular immune responses in different clinical manifestations of Chikungunya infection that would help in understanding the pathogenesis of CHIK infection.

Work done

Forty-six Chikungunya cases hospitalized with different clinical presentations (encephalitis cases, n=22, other systemic involvement (OSI) cases, n=12 and classical cases, n=12) were investigated. A control group consisting of seven age and sex matched healthy individuals negative for anti-CHIK IgM and IgG antibodies were also included. Confirmation of Chikungunya infection was assessed by the presence of IgM anti-CHIK antibodies and/or CHIK RNA detection. Seventeen of 46 cases had underlying medical conditions, six of them with more than 2 underlying medical conditions Underlying medical conditions were noted in 10 of 22 encephalitis cases, 5 of 12 OSI cases and 2 of 12 classical CHIK cases. CD4 T cell responses and the levels of pro and anti inflammatory cytokines against the envelope antigens (rE1p and rE2p) were assessed by lymphocyte proliferation assay and Cytometric bead array

Lymphocytes of 0/12 and 6/21 encephalitis cases, 1/6 and 4/12 other systemic involvement cases and 3/10 and 6/12 classical CHIK cases could recognize rE1p and rE2p respectively. Five of the six classical fever cases responding to rE2p were without any underlying medical condition at the time of Chikungunya infection. None of the 7 controls responded to rE1p and rE2p. **(Table 1)**

Table 1: CD4 T cell responses to rE1 and rE2 proteins in CHIKV infected individuals

Responder/Total tested (% Recall response)					
Encephalitis Cases (n=22)		Other Systemic Involvement Cases (n=12)		Classical CHIK Cases (n=12)	
Recall antigen		Recall antigen		Recall antigen	
rE1p	rE2p	rE1p	rE2p	rE1p	rE2p
0/11 (0%)	6/21 (28.5%)	1/6 (16.6%)	4/12 (33.3%)	3/10 (30%)	6/12 (50%)

Due to logistic problems, rE1p induced cytokine assays were carried out only in very few samples. Hence we are considering the results of only rE2p induced cytokines Figure 1 depicts cytokine levels in the PBMC culture supernatants in response to rE2 protein in different patient categories. The cytokine values are expressed in pico-gram as mean ± standard deviation. IL-5 levels from all the three patient categories were significantly higher than those in controls (4297.9 ± 1551.1 pg/ml in encephalitis cases, p<0.01; 4444.4 ± 1666.6 pg/ml in other systemic involvement cases, p< 0.01 and 3867 ± 994 pg/ml in classical fever cases, p=0.02, vs controls 4.9 ± 5.1 pg/ml). Difference in IL-5 levels among different patient categories were not statistically significant (p>0.05).

As compared to the controls (2.6 ±0.92pg/ml), IL-4 was significantly elevated in encephalitis cases (273.9± 166.1 pg/ml, p<0.01) and other systemic involvement (879.1± 1557.3 pg/ml, p<0.01) cases. IL-4 levels in the classical CHIK cases were also higher, though statistically not significant (468± 188 pg/ml, p=0.07**).** IFN-? levels in the encephalitis cases were significantly lower compared to controls (9.6 ± 8.3 pg/ml vs 383.6 ± 798.6pg/ml, p<0.01). No difference was recorded in IFN-? levels of the other two patient categories when compared with controls (p> 0.05). TNF-a**,** IL-10 and IL-2 levels among all cases and controls were comparable **(Fig. 2)**.

Overall, the present study suggests that infection with Chikungunya is associated with a Th2 biased cytokine response irrespective of the outcome and clinical manifestations and emphasizes need for in-depth studies in the pathogenesis of this re-emerging infection.

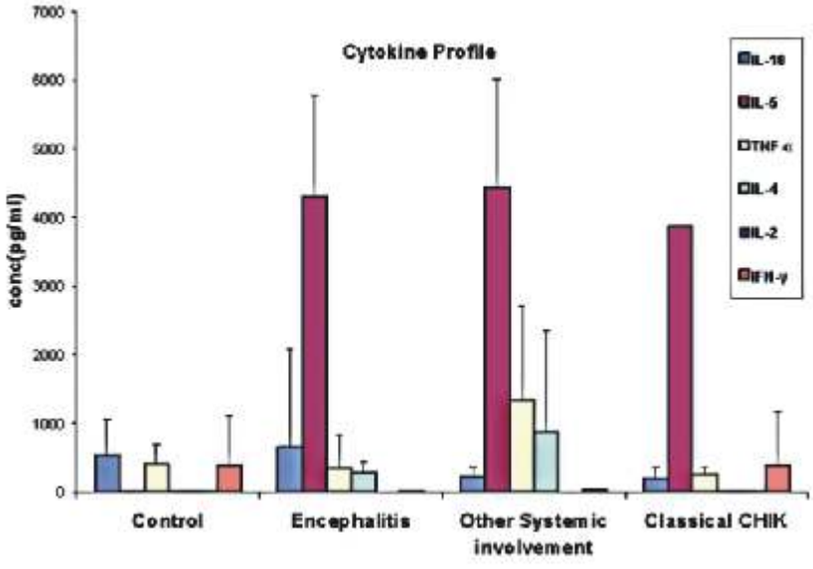


Fig. 2: Cytokine levels in culture supernatants of peripheral blood mononuclear cells after stimulation with CHIKV rE2p in different categories of Chikungunya patients & controls

Association of the neurological complications of the Chikungunya virus infection during the current epidemic with specific mutation(s) in the viral genome

VA Arankalle, SL Hundekar and SS Gandhe

Introduction

Re-emergence of Chikungunya (CHIK) in epidemic form has affected several countries from Indian Ocean islands and India. In addition to the predominant classical presentation, several complications of the infection have been recorded. Both host and viral factors play crucial roles in modulating the course of an infection. This study deals with the viral factors.

Objective

- To examine if the neurological complications of CHIK could be correlated with specific mutations in the viral genome.

Work Done

Full genomes of CHIK viruses from 5 cerebrospinal fluid (CSF)-derived isolates, one CSF and ~8.5 Kb genome from the other CSF were amplified, sequenced and compared with the sequences derived from viruses isolated from the classical cases. These sequences represented the states of Gujrat, Maharashtra and Karnataka. No specific mutations differentiating the two forms of the disease could be identified. Thus, the neurological complications of CHIK infection are not related to the specific mutations in the viral genome and in-depth studies in relation to the host factors need to be undertaken on priority.

Establishment and characterization of a new *Aedes aegypti* cell line for Chikungunya isolation and propagation

AB Sudeep, Deepti Parashar, VA Arankalle and AC Mishra

Introduction

The 2006-07 Chikungunya epidemic in the Indian subcontinent warranted the need for rapid diagnosis and isolation of the virus from field collected serum/arthropod specimens. Since CHIK is vectored by *Aedes aegypti* mosquitoes in India, it was felt necessary to establish a new cell line from the vector species in order to facilitate further studies on the virus at the cellular level.

Objectives

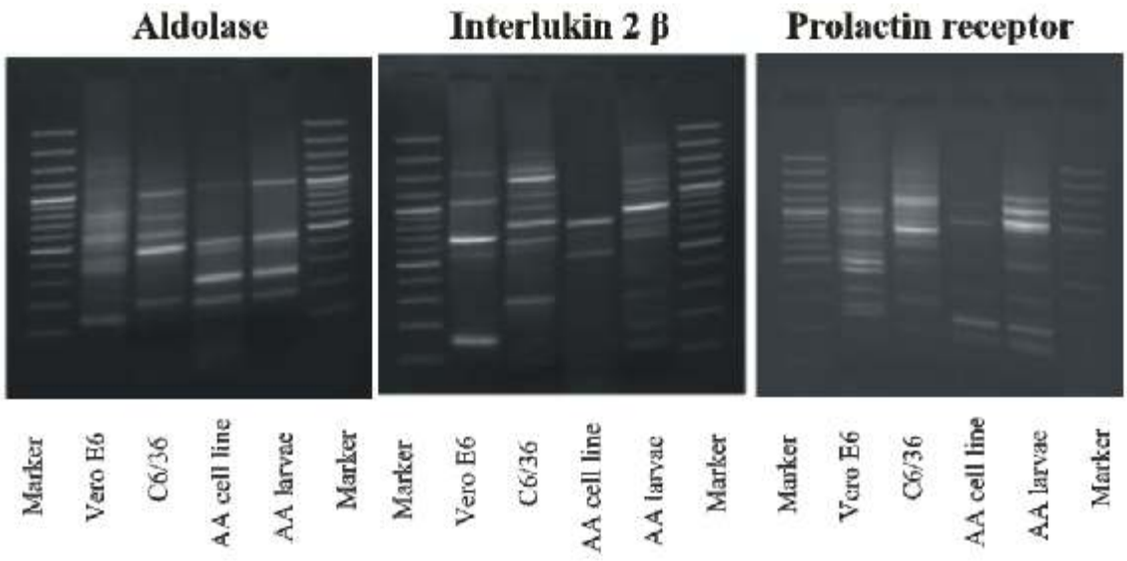
- To establish a new cell line from the vector species for CHIK studies
- To clone the cell line to isolate highly susceptible cell populations to CHIK virus
- Large scale propagation of CHIK virus antigen for diagnostic and vaccine studies.

Work done

A new cell line is established from *Ae. aegypti* mosquito from the neonate larvae and characterized. It is at the 62nd passage level and consists of three different cell types *viz*. epithelial like, fibroblast like and giant cells. The epithelial-like cells formed the majority of the cell population (>90%) and the other two cell types represent rest of

the population. Seventy-six percent cells showed diploid number (2n=6) of chromosomes at the 52nd passage level. The cells showed 10-fold increase in cell number at the 52nd passage level.

Species specificity of the cell line was determined using RAPD primers *viz*. mammalian aldolase, interleukin 2β and Prolactin receptor. The fingerprinting profile clearly demonstrated 100% homology with host insect confirming the origin of the cell line.



Susceptibility of the newly established cell line was studied and found that the cell line replicates (Japanese encephalitis (JE), West Nile (WN), Chikungunya (CHIK), Chittoor and dengue (DEN)) and two sandfly borne arboviruses (Chandipura and vesicular stomatitis virus). However, the cell line did not replicate two tick-borne arboviruses tested *i.e*. Ganjam and Kaisodi viruses. The virus yield of DEN, JE, WN, CHIK and Chandipura viruses in the cell line was very high and the cell line may find application in the detection, isolation and large-scale production of virus antigen.

Growth kinetics of Chikungunya virus in certain cell lines

AB Sudeep, VA Arankalle and AC Mishra

Introduction

The 2006-07 Chikungunya epidemic in the Indian subcontinent warranted the need for rapid diagnosis and isolation of the virus from field collected serum/arthropod specimens. Though many vertebrate and invertebrate cell lines supported the growth of the virus, it was felt necessary to determine the right cell line which is most sensitive and yield high titre for virus isolation and further studies.

Objectives

- To study the sensitivity and virus yield of different cell lines to CHIK virus

Work done

CHIK virus growth kinetics was studied in certain mammalian and insect cell lines to determine the virus yield. C6/36 cell line (a clone of Singh's *Ae. albopictus* cell line) was found the most sensitive and high yielding when a comparative study was carried out **(Fig. 3)**. Among the vertebrate cell lines, Vero E6 cell line yielded high titre of virus. Further studies with other cell lines are in progress.

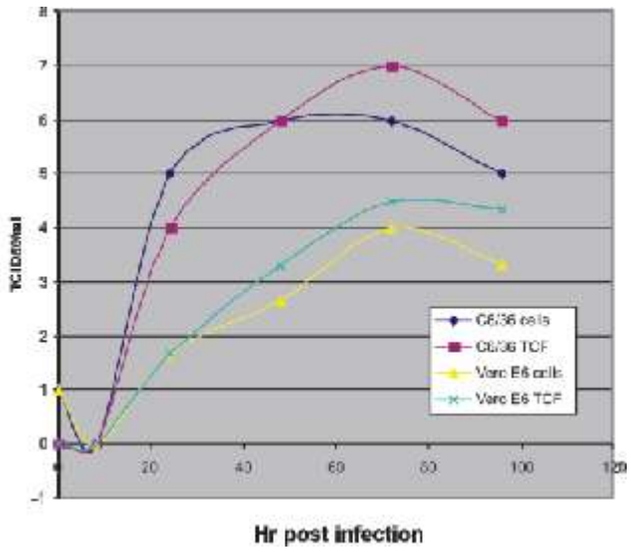


Fig. 3 : Growth of CHIK virus in Vero E6 and C6/36 cell lines

Diagnostic Activity

Despite providing a large number of diagnostic kits to various state laboratories, samples from suspected Chikungunya cases are received at the National Institute of Virology from all over India. These samples were screened for the presence of antiCHIK IgM antibodies in MAC-ELISA test (NIV Kit). Table shows the results of the tests carried out.

Chikungunya testing: 1 April 2007 to 31 March 2008

S. No.	State	Total samples	Chik IgM positive
1	Maharashtra	1682	391
2	Kerala	715	308
3	Gujarat	109	18
4	Pondicherry	02	00
5	West Bengal	14	07
6	Orissa	06	01
7	Tamilnadu	40	22
8	Madhya Pradesh	19	01
9	Delhi	06	02
10	Rajasthan	03	01
11	Karnataka	02	00
Total		**2598**	**751**

Molecular surveillance

Molecular surveillence for CHIK virus was continued. Two genomes from Kerala (2006 and 2007) were sequenced completely. In addition, from over 100 serum samples, small fragments of the genome were amplified and sequenced. These will be used for further analysis.

Publications

- Arankalle VA, Shrivastava S, Cherian S, Gunjikar RS, Walimbe AM, Jadhav SM, et al. Genetic divergence of Chikungunya viruses in India (1963-2006) with special reference to the 2005-2006 explosive epidemic. **J Gen Virol**; 2007 Jul; 88(Pt 7): 1967-76.

Workshops / Conferences / Seminar / Meetings attended

VA Arankalle

- Member of the Indian delegation to France, Presentation on "Chikungunya in India" at France (6-7th Jun 2007).
- WHO meeting of the Experts Group on 'Chikungunya Fever', at Aurangabad, Maharashtra (India) 27-29 September 2007.
- Invited speaker (Topic: Molecular epidemiology of Chikungunya in India.) during an International Workshop on "Molecular Epidemiology and Immunology of Malaria and other Vector Borne Diseases" at RMRC, Jabalpur, October 16-19, 2007.
- Invited speaker (Topic: Re-emergence of Chikungunya in India: Molecular studies.) at the Annual meeting of Indian Academy of Sciences at Trivendrum during 1-4 Nov, 2007.
- Presented multi-centric CHIK project to a special review committee at ICMR on 7th Nov 2007.
- Invited speaker (Topic: HEV Diagnosis" and "Chikungunya in India) at the International Conference on 'Emerging and Re-Emerging Viral Diseases of the Tropics and Sub-Tropics', at New Delhi during December 11-14 2007

Deepti Parashar

- Presented a paper entitled "Development of real-time RT-PCR for detection and quantification of Chikungunya Virus" in the International Conference Emerging and Re - emerging Viral Diseases of the Tropics and Subtropics, organized at IARI, Pusa Road, New Delhi from 11th -14th Dec. 2007.

AB Sudeep

Presented the following papers in the International Conference Emerging and Re - emerging Viral Diseases of the Tropics and Subtropics, organized at IARI, Pusa Road, New Delhi from 11th -14th Dec. 2007.

- Sudeep AB, Jadi RS, Basu A, Arankalle VA and Mishra AC. Growth kinetics of Chikungunya virus in certain cell lines.
- Jadi RS, Sudeep AB, Arankalle VA and Mishra AC. Inactivation kinetics of Chandipura with different agents with reference to immunogenicity.

Supriya Hundekar

- Presented poster titled 'Full Genome Sequence Analysis of Chikungunya Virus isolates From Kerala (India) during 2006-2007' at International Conference on 'Emerging and Re-Emerging Viral Diseases of the Tropics and Sub-Tropics', at New Delhi December 11-14, 2007.

Training programmes / Workshops / Seminars organized

The following persons were trained in animal and insect cell culture.

- Mrs. Rekha Jaiswal, under WHO fellowship undergone one month training in Animal Tissue culture.
- Mr. Chetan Mokashi (M.Sc.), Modern College, Shivajinagar has undergone a project entitled, "Characterization of insect cell lines" as partial fulfillment of M.Sc. degree of University of Pune.
- Ms. Archana Mokashe (M.Sc.), Annasaheb Magar College, Hadapsar has worked under Dr. AB Sudeep on a project entitled; "Growth and yield in *Helicoverpa armigera* NPV in *H. armigera* cell line under different nutritional and temperature conditions".
- Dr. Bharat Bhushan Sharma, from Indore Biotech, Madhya Pradesh has undergone a short-term training course on *in vitro* cultivation of NPVs in certain lepidopteran cell lines.

Human Influenza

Human Influenza

Scientific Staff

Dr. MS Chadha	Scientist E	mscniv@hotmail.com

Technical Staff

Mr. MS Pawar	Technical Officer
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Project staff

Ms. SR Waregaonkar	Research Assistant
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Mr. MS Rangole	Technician
Ms. SS Jagtap	Programmer
Mr. AS Salunke	Technician

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

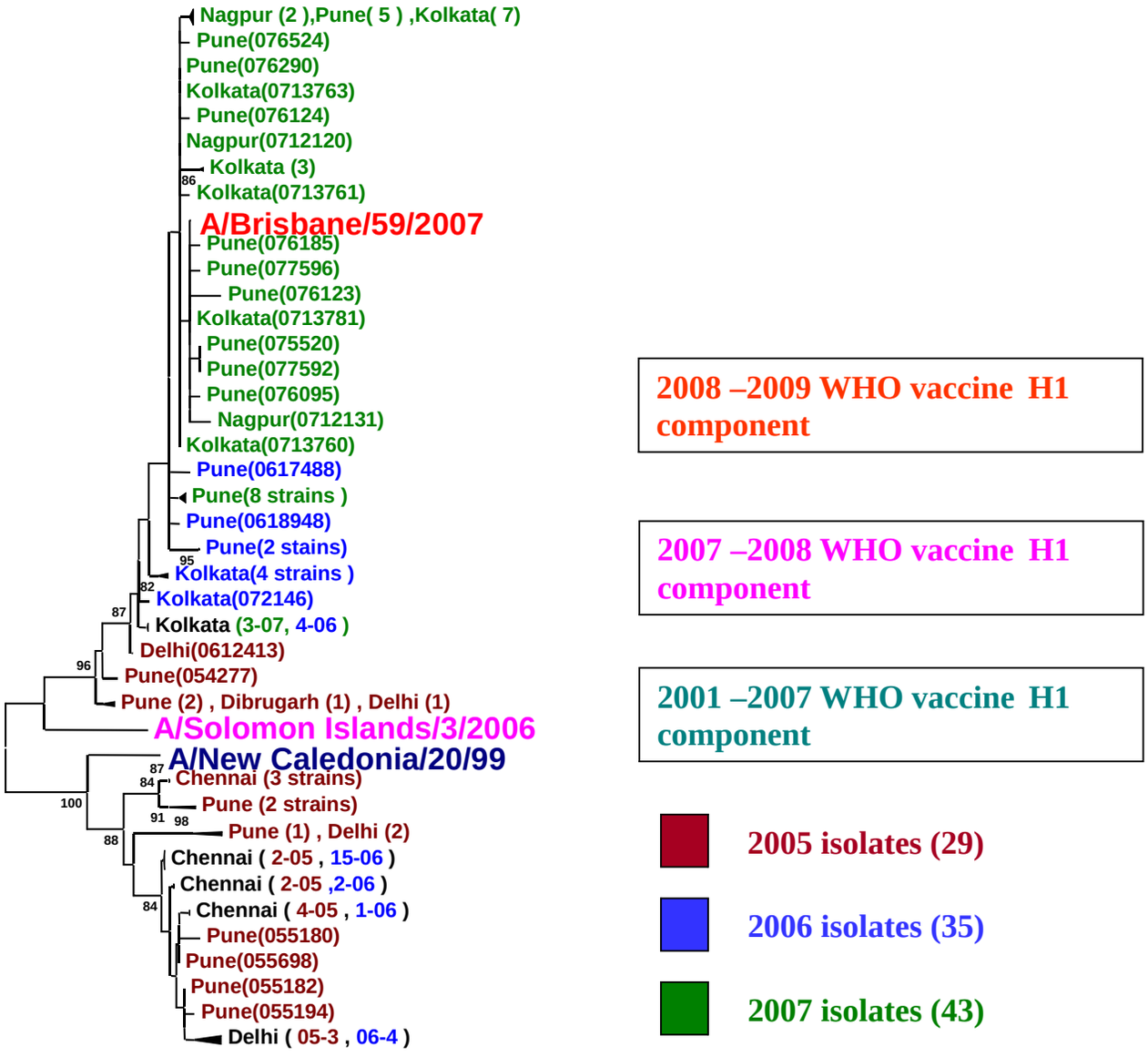

Fig 1. HA1 sequencing of 107 H1 isolates (979 bp)

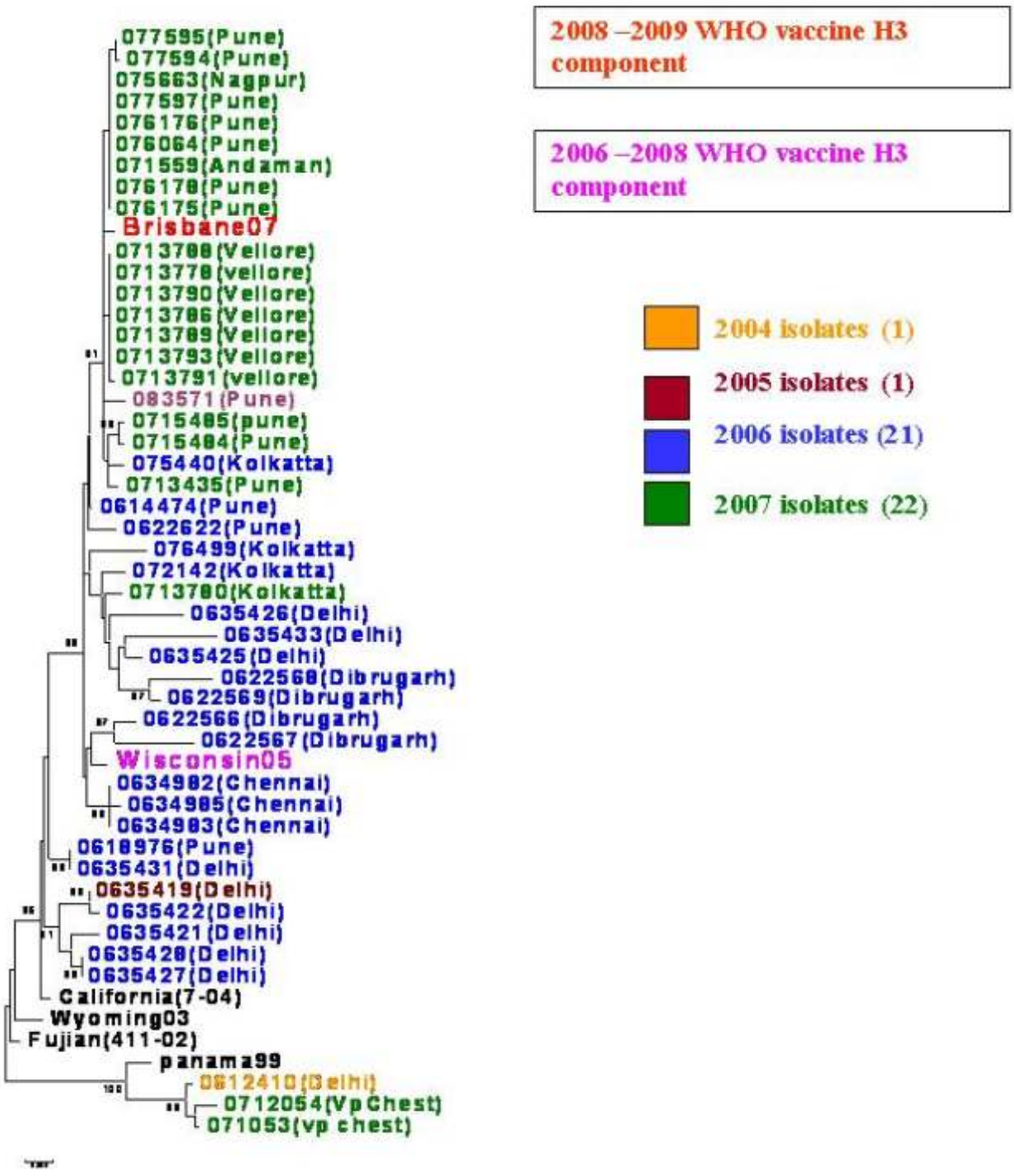

Fig. 2 : HA1 sequencing of 46 H3 isolates (984bp)

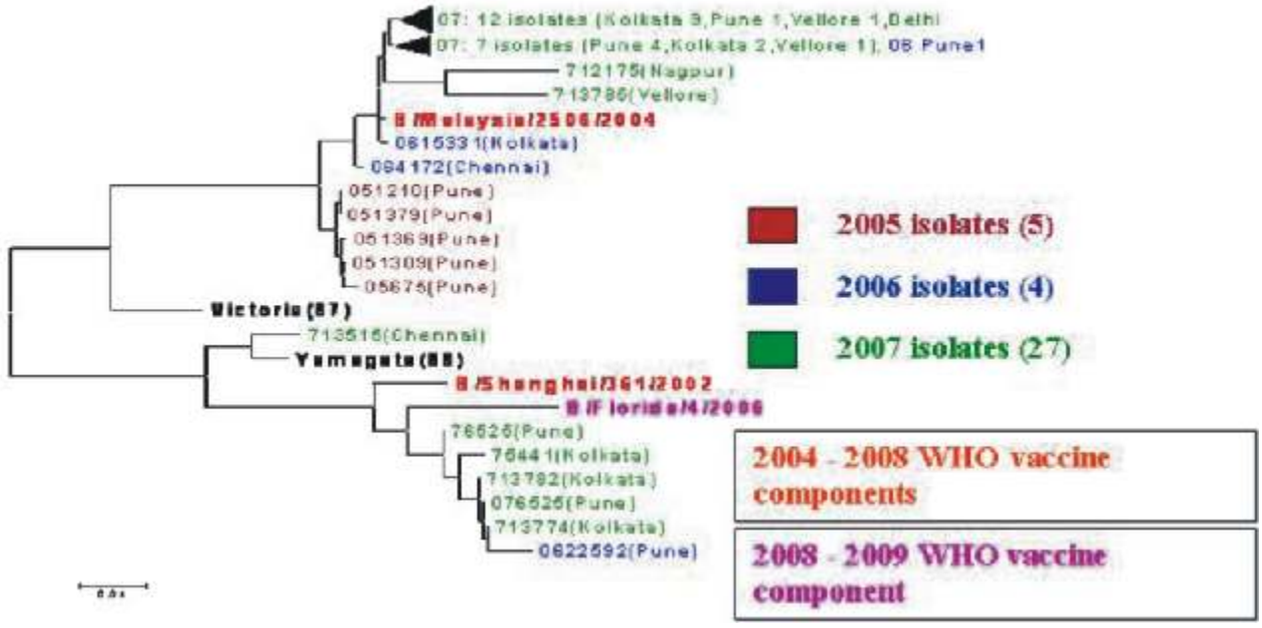

Fig. 3 : HA sequencing of 36 Type B isolates (855bp)

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 duel 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.

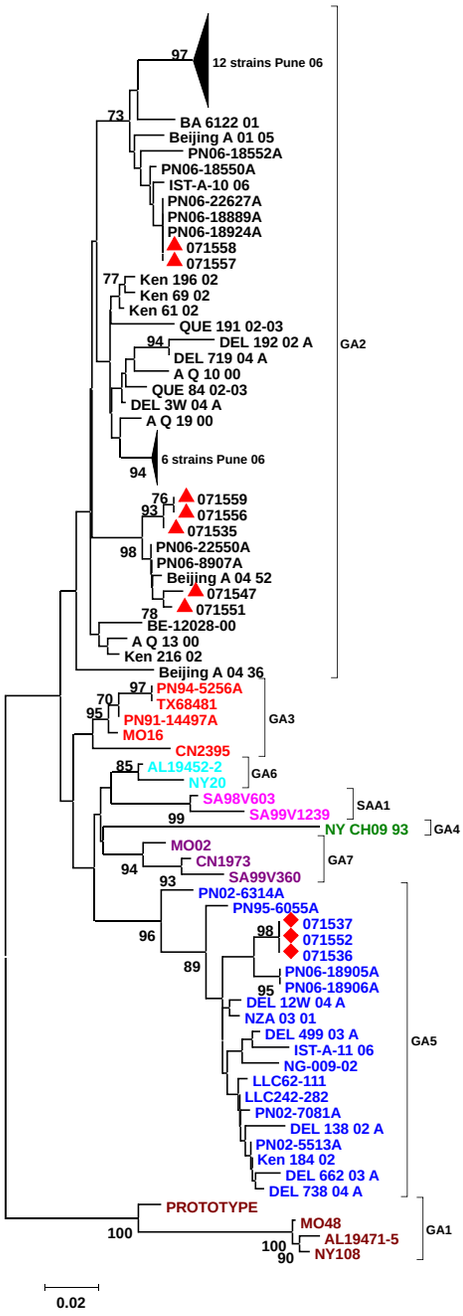

Fig. 5 : Phylogenetic analysis of HRSV strains using G protein gene

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)**.

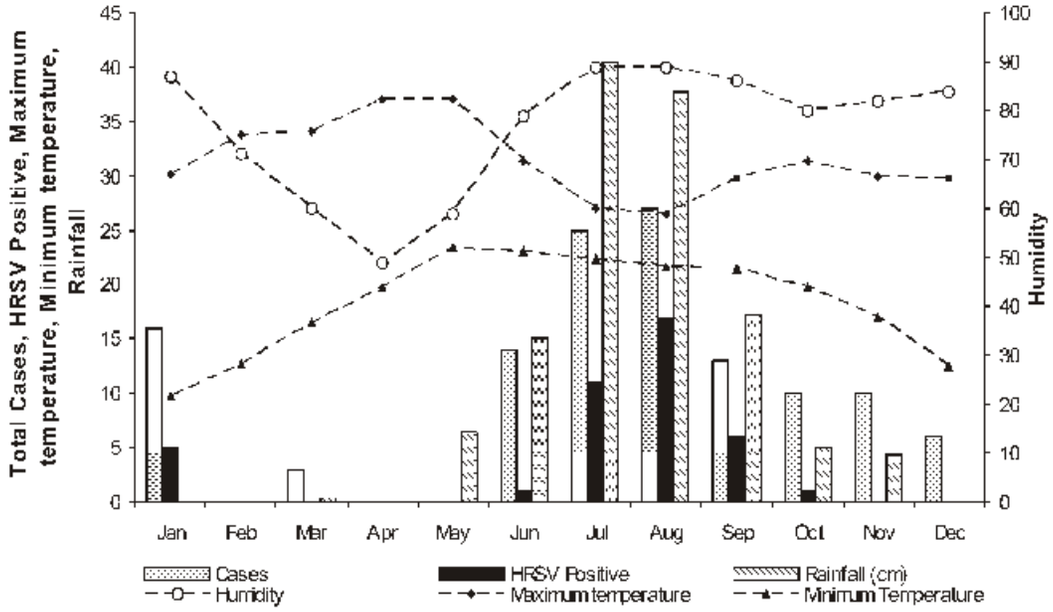

Fig. 6 : HRSV activity in relation to climatic conditions during 2006

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.

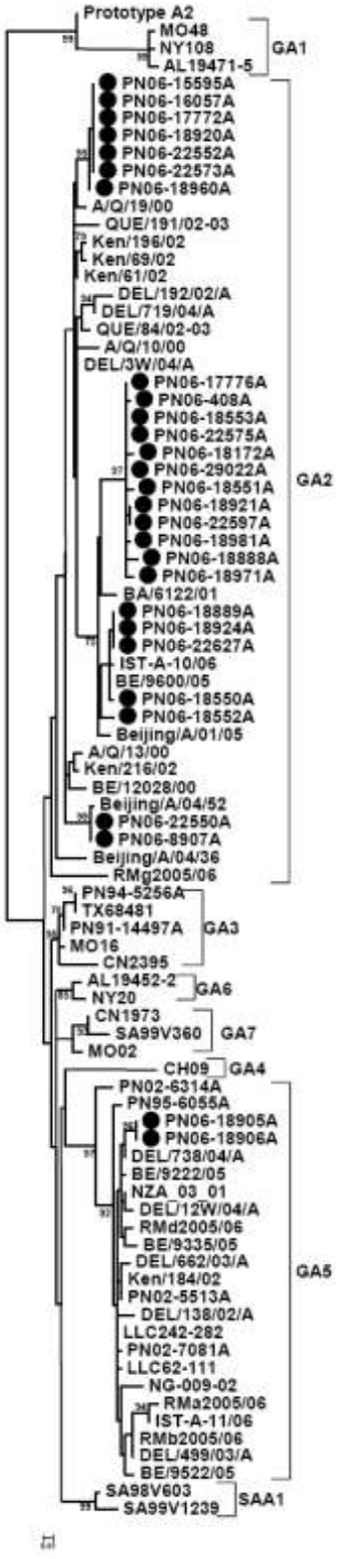

Fig. 7 : Phylogenetic tree of HRSV group A viruses.

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.

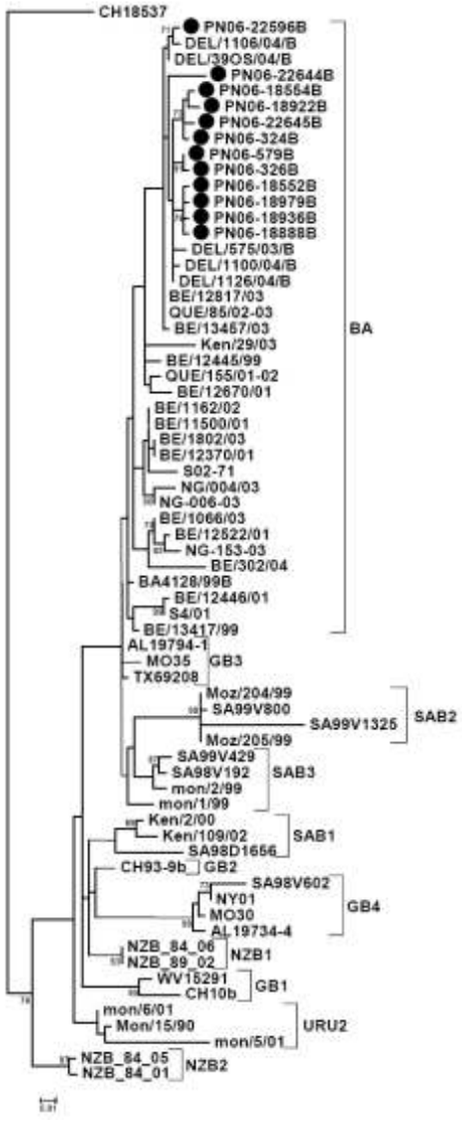

Fig. 8 : Phylogenetic tree of HRSV group B viruses

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 nasopharengeal 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 nd 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.

Avian Influenza

Avian Influenza

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Mr. Vishal Thite	Technician

8 Avian Influenza

- Avian Influenza Outbreak Investigations
- Avian Influenza surveillance during avian migratory season 2007-2008
- Seroprevalence of antibodies to influenza A viruses using Microneutralization (MN) & Hemagglutination inhibition (HAI) assays in human sera from Nandurbar & Jalgaon districts, India
- Generation of infectious influenza A virus particle and vaccine reference strain of highly pathogenic influenza viruses (H5N1) using reverse genetics

Avian Influenza Outbreak Investigations

Virological Investigation Group : SD Pawar, AK Chakrabarty, K. Ray, SS Koratkar, SS Kode, B. Pal, S. Raut, VV Thite, MR Khude, D. Hangekar.

Molecular Detection and Characterization Group :

- **West Bengal Outbreak :** AK Chakrabarti, SD Pawar, S Raut, B Pal
- **Manipur Outbreak :** K Ray, AK Chakrabarti, SD Pawar B Pal, S Raut

Along with AI group, staff members from the following groups were also involved in the outbreak investigation. Processing of bird specimens: DR Patil, P Barde, Sandeep, RS Jadi, and Dinesh Singh. RT-PCR & Sequencing: Hepatitis, Japanese Encephalitis, Measles, Rotavirus, Dengue

Introduction

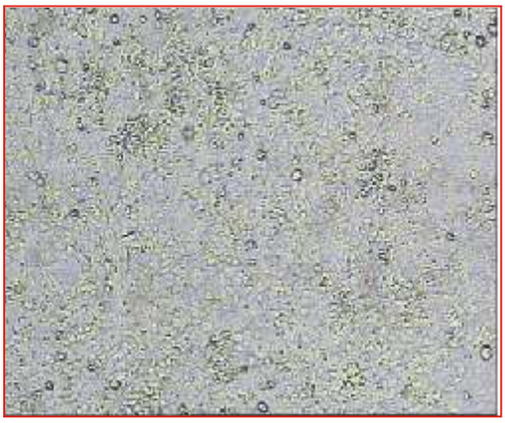
The recent emergence of Avian Influenza and specifically the subtype H5N1 pose a major problem and a biggest threat to public health globally as well as in India. H5N1 virus is known to cause widespread infection in birds and also to human respiratory tract. It is very important to identify this lethal virus if it is present in any outbreak. We established a combination of molecular diagnostics, serological tests and virus isolation to identify and confirm the presence of highly pathogenic Avian Influenza viruses from field/clinical specimens. During the last year India experienced outbreak of H5N1 in poultry in several occasions Manipur (July 2007), West Bengal (January to April 2008). We received samples from the entire above outbreak area and investigated to confirm the presence of highly pathogenic avian influenza viruses (H5N1) in these samples.

Objectives

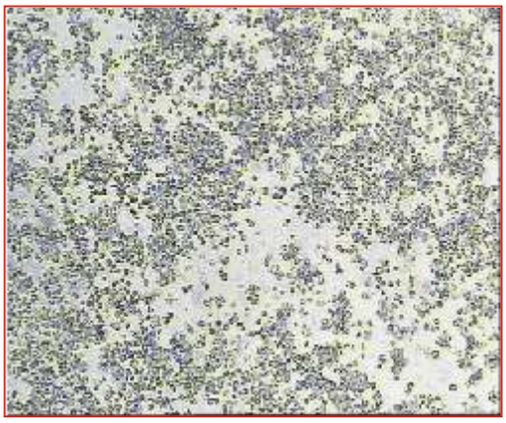
- Virus isolation and characterization of H5N1 isolates.
- Molecular detection and characterization of H5N1 strains.

Work done

Specimens received from outbreak areas were processed in high containment laboratory for virus isolation in SPF eggs and MDCK cell line. We performed one step reverse transcription-polymerase chain reaction (RT-PCR) using WHO sets of diagnostics primers specific for influenza A, HA (H5) and NA (N1) and Real time RT-PCR using ABI influenza A/H5 and N1 kits. Surface glycoproteins (Hemagglutinin and Neuraminidase) were sequenced from all the isolates. The specimens which were positive for H5N1 were further processed for virus isolation in 10-day-old embryonated chicken eggs and in MDCK cell line. Chick embryos died within 24 hours post-infection. Madin-Darby Canine Kidney (MDCK) cultures with confluent monolayer were prepared. These bottles were inoculated and observed for cytopathic effect (CPE) (**Fig. 1**). CPE was evident within 48 hours post-infection. When +++ CPE was evident, TCFs from these bottles were harvested and tested by HA test for virus titration. Characterization of the genome of Manipur and West-Bengal strains have been performed for all the 8 gene segmentsof representative isolates of Manipur and West-Bengal. Full genome sequencing revealed the presence of unique virus, which belongs to clade 2.2 of Z genotype with few unique mutations. Additionally, molecular marker analyses also depicted that this virus is sensitive to drugs Amantadine and Oseltamivir. All the above studies confirm the virus to be of avian origin.



Un-inoculated MDCK cells.



CPE on MDCK cell culture, induced by influenza H5N1 Manipur (MNI 1900)

Fig. 1 : Cytopathic effect of Manipur H5N1 virus in MDCK cell line

Allantoic fluids and MDCK supernatants were positive in hemagglutination (HA) tests with fowl and horse RBCs. Representative isolates were tested in hemagglutination inhibition (HAI) test for identification of viruses. A panel of antisera used for identification included antibodies against influenza A(H5N1)-Navapur A/Ck/India/33487/2006-H5N1, (H5N2), A(H7N3), A(H9N2), NDV and normal ferret and sheep sera. These isolates were identified as influenza H5N1.

AI surveillance during avian migratory season 2007-2008

SD Pawar, SA Pande, AK Chakrabarti, SS Koratkar, SS Kode, VV Thite, MB Nanavare, B. Pal, S. Raut, MR Khude, AV Jamgaonkar, P. Salunke, DS Hangekar and ELA foundation team.

Introduction

India reported outbreaks of HPAI H5N1 in poultry in Navapur, Maharashtra; Gujarat; Madhya Pradesh (February 2006), Manipur (July 2007), West Bengal (January 2008). The role of migratory birds in the transmission of H5N1 viruses is still unclear. In this scenario Avian Influenza (AI) surveillance in wild migratory, wild resident, domestic birds and poultry was undertaken by NIV jointly with ELA Foundation, Pune, India during 2006-2007.

Objectives

- Surveillance of H5N1 or any avian influenza in migratory and wild birds, and poultry
- Characterization of the strain found in the surveillance.

Work done

AI surveillance in migratory and wild birds was undertaken during avian migratory season 2007-2008 in collaboration with ELA foundation, Pune. During this season, 678 specimens (Fecal droppings: 3300, Cloacal swabs: 22, Tracheal swab: 12, Blood sample: 5) were collected from sites covering Maharashtra and Karnataka state. Screening of these specimens using molecular and virological diagnostics such as one-step RT-PCR, Real-Time PCR and virus isolation was performed. All these specimens were inoculated in embryonated chicken eggs for virus isolation.

A total of six fecal specimens from Eurasian Spoonbill, Shoveller, purple moorhen, Asian Openbill, Great Thick Knee or Greater Stone Plover and Greater Flamingo were positive in hemagglutination (HA) assay. These HA

positive allantoic fluids were tested in quick tests for influenza A, H5, NDV and IBDV. Specimen from Eurasian Spoonbill collected from Rui Chatrapati, Ahemednagar district was positive for influenza A. Further analysis of this specimen by RT-PCR and sequencing showed that this virus is of influenza A (H11N1) subtype. This is the first ever influenza A (H11N1) virus from the Indian subcontinent.

All other specimens were negative for influenza A, H5, NDV and IBDV. Electron microscope analysis of two representative specimens showed presence of Reovirus-like particles. Further analysis of these specimens is in progress.

Seroprevalence of antibodies to influenza A viruses using Microneutralization (MN) & Hemagglutination inhibition (HAI) assays in human sera from Nandurbar & Jalgaon districts, India

(Work done at Centers for Disease Control and Prevention (CDC), Atlanta, USA)

Personnel involved in the investigation: SD Pawar, Scientist B, Avian Influenza visited and worked as a "Guest Researcher" at CDC, Atlanta, USA.

Field study & sampling by: NS Wairagkar, MS Chadha, CG Raut.

Supervisor: Jacqueline Katz, Chief, Immunology & Pathogenesis Branch, Influenza Division, CDC. Work was done with Jenna Achenbach and Veguilla Vic.

Introduction

India reported outbreaks of HPAI H5N1 in poultry in Navapur, Maharashtra; (February 2006) and NIV had sequenced H5N1 isolates. In order to understand the seroprevalence of antibodies to influenza A in the human sera two different assays were performed. The microneutralization (MN) assay is sensitive and detects H5-specific Ab in human serum specimens at low titers. The haemaglutination inhibition (HAI) assay is advantageous over the MN assay as the assay works well with inactivated H5 viruses, so that the A (H5N1) serology can be safely performed in a BSL-2 laboratory.

Objectives

- Detection of antibodies to AI A (by MN assay and HHAI assay)

Work done

A total of 100 test sera and 25 sera from unexposed population were tested in MN assay. It was found that all the sera were negative for the presence of antibodies against an Indian isolate of influenza A (H5N1) virus. However, these sera showed presence of Abs against human influenza A (H1N1) [62.4 % sera positive] & A (H3N2) [88.8 % sera positive] strains. Cut-off in the test was antibody titer >=80. Serum specimens with A (H5N1) viruses were tested using 1% horse RBCs and with human influenza A (H1N1) and A (H3N2) viruses were tested with 0.5 % turkey RBCs for HAI assays. All the tested sera were negative with Indian isolate of influenza A (H5N1) virus and with A/Whooper swan/Mongolia/244/2005 (H5N1) virus. However, these sera showed presence of Abs against human influenza A (H1N1) [20.8 % sera positive] & A(H3N2) [78.4 % sera positive] strains. Cut-off in the test was antibody titer >=40.

Generation of infectious influenza A virus particle and vaccine reference strain of highly pathogenic influenza viruses (H5N1) using reverse genetics

AK Chakrabarti, B. Pal, S. Raut and AC Mishra

Introduction

Reverse genetics is one of the most important and latest tools that have revolutionized the research in influenza virus to generate recombinant virus, vaccine virus strain and study pathogenicity.

Objectives

- Construct a pre-pandemic vaccine reference strain from highly pathogenic avian influenza virus (H5N1) of Indian origin.

Work done

Reverse genetics facility was set up for the rescue of influenza virus in collaboration with St. Jude Children's Hospital, Memphis, TN, USA. We have successfully rescued wild type A/WSN/33 and currently working to rescue wild type influenza A (H5N1) viruses. Cloning of different gene segment of highly pathogenic avian influenza viruses is underway.

The highly pathogenic AI strain A/Chicken/India/NIV-33498/06 (H5N1) was modified to a low pathogenic virus using recombinant DNA technology and reverse genetics. Recombinant virus generated possessed surface glycoproteins (modified HA and complete NA) of influenza A H5N1 in A-PR8 background and grew well in embryonated chicken eggs to a HA titer of 1256. This is a low pathogenic virus as per its dependency on trypsin for plaque formation and animal challenge study and can be used to generate inactivated vaccine against highly pathogenic avian influenza A (H5N1) viruses.

Modification of the above HPAI virus was performed at CDC, Atlanta. AK Chakrabarti[1], Y Matsuoka[2] and RB Donis.

Future Plan

- Characterization of the strains of H5N1.
- Initiate studies on animal models for H5N1.
- Avian influenza surveillence in India and study at the avian-human interface.
- Development of antiviral agent.

Publications

- 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.

Book Chapter

- Shailesh Pawar "Avian Influenza", Lecture notes for Certificate Course in Basic Ornithology. Edtrs. S. Pande, S. Kharat, H. Ghate, A. Mahabal; Published by MES Abasaheb Garware College & ELA Foundation, Pune, 2008, p225 - 233.

Workshop/Conference/Meeting Organized

- AI Group organized an International Workshop in collaboration with CDC, USDH on Real-Time PCR for detection of AI viruses, 8-12, Oct. 2007.

Workshops / Conferences / Seminar / Meetings attended

SD Pawar

- Invited talk in International symposium on AI, New Delhi 28-31 Oct. 2007, "Neutralizing antibodies as a surveillance tool for AI".
- Invited Lecture on Avian influenza surveillance in migratory birds, International Workshop on Avian influenza surveillance, organized by BNHS-FAO-Wetlands International organization, 8th February 2008, Nashik.
- Attended meeting on Options for the control of influenza VI at Toronto, Ontario, Canada, June 17- 23, 2007. A discussion between Indian scientists and CDC Atlanta influenza group was arranged by the organizing committee of the "Option for the control of Influenza VI" Conference held in Toronto, Canada. Presented the work carried out by him at CDC Atlanta with respect to Microneutralization (MN) assay for detection of antibodies against avian influenza (AI) A (H5N1) virus and Haemagglutination (HAI) assay using horse RBCs.
- Presented data of the work performed at influenza branch, CDC to the staff of influenza division, CDC, June 11, 2007.
- Invited Lecture on Avian influenza, Ornithology course, Abasaheb Garware College, Pune, 14th January 2008.

Posters presented in an international ICVT conference, New Delhi, 11-14 Dec. 2007.

- Development of HA & HAI assays for detection & identification of AI viruses. Pawar SD, Koratkar SS, Thite VV, Kode SS, Khude MR, Nanaware MB, Mishra AC.
- AI surveillance in migratory and domestic birds during avian migratory season 2006-2007. Pawar SD, Pande SA, Koratkar SS, Kode SS, Thite VV, Nanavare MB, Khude MR, Randive SN, Jamgaonkar AV, Ray K, Mishra AC.

K Ray

- Attended meeting on Options for the control of influenza VI at Toronto, Ontario, Canada, June 17, 2007 to June 23, 2007

AK Chakrabarti

- Attended meeting on Options for the control of influenza VI at Toronto, Ontario, Canada, June 17, 2007 to June 23, 2007. Delivered a talk entitled "Development of PR8 reassortant vaccine strain against A/Chicken/India/NIV-33498/06 (H5N1)" that was carried out at CDC, Atlanta. Discussion session with Dr. Erich Hoffmann of St. Jude Children's Hospital, Memphis, TN, USA regarding future collaboration.
- Invited talk in an international symposium on AI, New Delhi 28-31 Oct. 2007, Title: "Reverse Genetics Modified Indian H5N1". Worked as a repertoire for a session in the same symposium.
- Guided our team in Real-Time PCR Training at Applied Biosystems authorized training center at Gurgaon (Lab India).
- Participated in CDC workshop "Real Time PCR detection of Avian Influenza virus" at NIV, 8-15 November 2007. Worked as a faculty to facilitate the workshop.

- Involved in teaching M.Sc. virology students.

Santosh Koratkar

- International Symposium on Avian Influenza: Epidemiological, Basic and Applied Research, Oct. 29-31, 2007.
- Training Attended workshop on Real-Time detection of Human and Avian Influenza, at NIV Pune, 8-15 November 2007.

Sadhana Kode

- Conference Attended and Presented Poster- International Conference on Emerging and Re- Emerging Viral Diseases of the Tropics and Sub- Tropics, December 11-14, 2007, New Delhi.

Biswajoy Pal

- Real- Time PCR Training at Applied Biosystems authorized training center at Gurgaon (Lab India).
- Participated in CDC workshop “Real Time PCR detection of Avian Influenza virus” at NIV, 8-15 November 2007.
- Attended international conference “ICVT' 07 at ICAR, New Delhi, presented principal authored poster at the conference.

Satish Raut

- Real- Time PCR Training at Applied Biosystems authorized training center at Gurgaon (Lab India).
- Participated in CDC workshop “Real Time PCR detection of Avian Influenza virus” at NIV, 8-15 November 2007.

Vishal Thite

- Conference Attended and Presented Poster- International Conference on Emerging and Re- Emerging Viral Diseases of the Tropics and Sub- Tropics, December 11-14, 2007, New Delhi.

Enteric Viruses

Enteric Viruses

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Mr. MM Yadav	Senior Research Fellow

9 Enteric Viruses

- Epidemiological studies on Rotaviruses
- Full length genome sequencing and characterization of rotavirus strains
- Molecular characterization of rotavirus strains adapted to cell culture
- Development of an ELISA for detection of rotavirus serotype specific antibodies in human sera and its evaluation for diagnosis of acute rotavirus infection
- Preparation of egg yolk antibodies against human rotaviruses
- Hospital based surveillance of non-rota enteric viruses in acute gastroenteritis patients
- Additional Studies
  Investigation of Outbreaks of Acute Hemorrhagic Conjunctivitis (AHC) occurred in Maharashtra and Karnataka states during 2006 and 2007

Epidemiological studies on Rotaviruses

Hospital based surveillance of rotavirus disease and strains among children

SD Chitambar

Rotavirus infections are the major cause of severe dehydrating diarrhea among children. Rotavirus serotypes G1P[8], G2P[4], G3P[8] and G4P[8] are most commonly circulating types globally. In addition to these, uncommon and untypeable rotavirus strains cocirculate and mixed infections with different rotavirus types occur in developing countries. In order to have better understanding of such viruses, epidemiological and molecular studies are required that would help to define the need for and benefits of rotavirus vaccines in India.

Objectives

- To estimate the proportion of rotavirus diarrhea
- To find out prevalent rotavirus types among hospitalized children <5 years of age

Work done

Fecal specimens were collected from a total of 284 children <5 yrs of age, hospitalized for diarrhea. Nearly 38.4% specimens were detected positive for rotavirus by ELISA. Among these specimens, 97.2% were typed for VP7 (G) and 98.1% were typed for VP4 (P) genes. Both G and P types were established in 96.3% of the specimens by multiplex PCR. Each of the two common rotavirus types G1P[8] and G2P[4] represented 57.2% and 17.1% of the strains respectively while none of the specimens showed presence of other two common types-G3P[8] and G4P[8]. G12P[8], G9P[8], G10P[8] and G12P[6] were detected at a frequency of 0.9%, 1.9%, 1.9% and 6.6%, respectively. G and P types in uncommon combinations (G1P[4], G2[8]) and in mixed infections represented 4.7% and 11.4% of the strains respectively (**Fig. 1**). A very few of the strains remained either G or P untypeable (0.9%-1.8%).

Hospital based surveillance of rotaviruses was also carried out in acute diarrhea patients from Jabalpur (n=84). Rotavirus positivity was detected in 10.7% of the patients. G1P [8] was detected in 66.6% whereas G2 P [4], G9 P [4] and G12 P [6] were detected each at 11.1%.

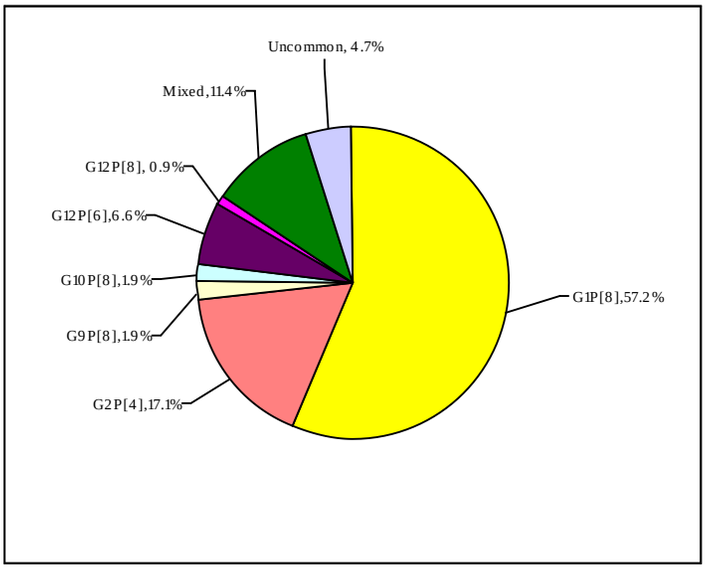

Fig. 1 : Distribution of rotavirus G and P types among typeable specimens

Future Plan

The work will be continued further to analyze the data on rotavirus serotypes and strains and their variations, untypeable strains and seasonal distribution of rotavirus associated hospitalizations.

Rotavirus viremia in patients with acute gastroenteritis

SD Chitambar

Thirty One pairs of stool -serum specimens collected from hospitalized diarrrheal patients during Nov. 2004- Feb. 2005 were investigated for rotaviruses by RT-PCR using VP6 gene primers and tested in ELISA for rotavirus antigen and anti-rota IgM antibody.

Rotavirus RNA was detected in 80.6% stool and 58.1% sera as against rotavirus antigen in stool (45.2%) and anti-rota IgM antibody (25.8%). All PCR positive specimens were typed for VP7 and VP4 genes by multiplex PCR. Five of 16 pairs could be typed for both the genes. Three of 5 pairs showed correlation between genotypes from stool and serum samples (G2P[4] vs G2P[4]) while two showed discordance (G2P[4] vs G12 P[8]; G2P[4] vs G8P[4]). Though a significant number of sera (56.3%) remained untypeable, data indicating seropositivity of rotavirus in gastroenteritis patients may influence the surveillance studies based on fecally excreted rotavirus strains and contribute to reveal dual/mixed infections in acute gastroenteritis patients.

Detection and characterization of rotaviruses in adolescent and adults cases of acute gastroenteritis

VS Tatte and SD Chitambar

Rotaviruses are the major cause of acute gastroenteritis in children. These viruses are also known to infect adolescents and adults. Recent studies carried out among adults in the developing as well as developed countries have shown rotavirus infections to be on the rise. Limited studies conducted so far in adults from India indicate 5-7% prevalence of acute rotavirus infections, however no data is available on molecular characteristics of rotavirus strains circulating in adolescent and adult cases of acute gastroenteritis.

Objectives

- To characterize the rotavirus strains recovered from adolescent and adult cases of diarrhea.

Work done

Two hundred and fifty three stool specimens were collected during 2004-2007 from adolescent and adult cases visiting OPD of local hospitals for acute gastro enteritis. Fourty four samples (17.4%) positive in ELISA for group A rotavirus were tested in monoclonal antibody based ELISA to determine the subgroups (SGs) of rotavirus strains. Results indicated subgroup diversity with predominance of Non I Non II SG followed by SGI+II, SGI and SGII in rotavirus strains **(Table 1)**. In comparison with the data reported earlier on such strains (AR 2001), a rise in NonI Non II SGs was noted.

Table 1 : Distribution of SGs in rotavirus strains

SG I	SG II	SG I + II	SG II
11.4% (5/44)	4.5% (2/44)	31.8% (14/44)	52.3% (23/44)

Among sixty seven stool specimens collected in the year 2007 from adolescent and adult cases of acute gastroenteritis, 31(46.3%) showed the presence of Group A rotavirus by ELISA. VP7 and VP4 genes of rotavirus strains were further characterized by multiplex PCR. Nearly 20% (6/31) of the specimens were typed for both VP7 and VP4 genes. All strains (n=6) that were typed for both the genes showed unusual combinations of G and P types (3/6 G1P[4],1/6 G9P[4], 1/6 G9P[6] P[4], 1/6 G1,G2 P[4]). Dual P type infections were detected in 6/9 of the P typed specimens. Majority of the strains 15/31 remained untypeable. Unusual / untypeable strains also contributed to subgroup untypeability of rotavirus strains.

Group A rotavirus ELISA negative samples (n=215) obtained during winter months of 1994-95 from adolescent and adult cases of acute gastroenteritis admitted to Naidu Hospital, Pune were screened for groups A, B and C rotavirus RNA by PAGE. Five samples (2.3%) detected positive, showed long RNA migration pattern. Multiplex PCR based characterization showed presence of G3P[8] in 2, G1P[8] in 2 and G nontypeable P[8] in 1 of the specimens. G3P[8] was detected for the first time in adult cases from Pune , western India.

Detection and molecular characterization of rotaviruses from cattle

SD Chitambar and CG Raut

Group A rotaviruses, members of the genus *Rotavirus* within the family *Reoviridae*, are the main pathogens of neonatal calf diarrhea and are classified into G and P genotypes. Among the 19 G and 27 P genotypes, ten G (G1,G2,G3,G5-G8, G10, G11 and G15) and six P(P[1], P[5], P[11], P[14], P[17] and P[21]) genotypes have been associated with diarrhea in calves, and of them, G6,G8 and G10 in conjugation with either P[1], P[5], and P[11] are of epidemiological significance.

Objectives

- To detect rotaviruses from apparently healthy and diarrheic cattle
- To characterize bovine rotaviruses by RNA-PAGE, RT-PCR and sequencing

Work done

A total of 78 fecal specimens collected from cattle were investigated for rotaviruses. Bovine rotavirus prevalence was 3.8% by ELISA. All ELISA positive specimens (n=3) were amplified in RT-PCR for VP7 and VP4 genes. Sequence analysis of PCR products revealed presence of novel G8P[14] strains closely related to bovine B17 strain of G8 type with 93.9-98.2% and human Hun 5 strain of P[14] type with 94.7-95.4% nucleotide identity. The data suggested the possibility of reassortment between human and bovine group A rotaviruses.

Full length genome sequencing and characterization of rotavirus strains

SD Chitambar and PR Fadnis

The rotavirus strain G2P[4] circulating commonly and G12P[6] emerged recently were undertaken for full length genome sequencing. The sequences obtained from 6 structural and 5 nonstructural genes were compared with reference strains. G2P[4] strain showed 84.4-92.6% and G12P[6] strain showed 96.7-99.0% nucleotide identity with reference strains TB-Chen and Dhaka 12-03 respectively.

Molecular characterization of rotavirus strains adapted to cell culture

Ritu Arora and SD Chitambar

Rotaviruses, members of the family Reoviridae, are the most common cause of severe diarrhea among children. Characterization of rotavirus genes especially of VP7 and VP4 from wild type rotavirus strains recovered from fecal specimens is widely carried out. However**,** limited studies are reported on the genomic changes that occur in human rotavirus during cell culture adaptation**.**

Objectives

- To isolate rotavirus strains from acute diarrhea patients
- To study the genomic changes in rotavirus during adaptation to cell culture

Work Done

Studies conducted earlier on isolation of rotavirus strains from acute diarrhea patients were continued further. Presence of a rotavirus strain in the infected MA-104 cells was confirmed by antigen capture ELISA. RT-PCR followed by sequencing for VP6 gene was carried out at passage 5. The strain showed 98% identity with Matlab13-03 strain (Bangladesh). RT-PCR and sequencing were carried out for all the structural (VP1-VP4, VP6 & VP7) and non structural (NSP1-NSP5) genes of wild type and culture adapted (at passage 5 and passage 10) strains. Sequences were compared with prototype strain, KU from Japan. Substitution was noted only in the deduced amino acid sequence of VP4 gene. All the genes of a culture adapted strain at passages 5 and 10 revealed 100% identity at nucleotide and amino acid levels.

Development of an ELISA for detection of rotavirus serotype specific antibodies in human sera and its evaluation for diagnosis of acute rotavirus infection

SD Chitambar, PG Ray and S. Bhalla

Rotaviruses are recognized as the most important etiologic agents of childhood diarrhea, worldwide. The widespread distribution of rotavirus is indicated by universal acquisition of serum antibodies to rotaviruses at an early age. A high prevalence of rotavirus antibodies in the adults suggest that repeated infections occur.

Objectives

- To develop an ELISA for detection of rotavirus serotype specific antibodies in human sera
- To determine the prevalence of rotavirus serotype specific antibodies in healthy adults and acute diarrhea patients
- To determine the correlation between serotype specific antibodies in serum and infecting genotypes in fecal samples of acute diarrhea patients

Work done

Results on rotavirus serotype specific antibodies in serum samples of healthy adults by a newly developed rapid assay (blocking ELISA) and comparison of the results with that of conventional tissue culture based neutralization assay have already been provided in the previous report (Annual Report 2006-07). Briefly, antibody titers to

different human rotavirus serotypes G1-G4 and animal strains G6 (Bovine) and SA-11 (Simian) were determined in 37 healthy adult sera by blocking ELISA. Fifteen of the 37 sera were subjected to the routine neutralization assay based on tissue culture and ELISA. No significant difference in percent positivity as well as range of titers was observed by both the methods.

For further evaluation of blocking ELISA, 14 acute and 9 convalescent phase serum samples of children hospitalized for rotavirus diarrhea were tested for serotype specific antibodies. Simultaneously, fecal specimens from the respective children were also tested for the infecting G-type by multipex PCR. Two to 4-fold rise in the blocking ELISA antibody titers was observed in all the 9 convalescent phase sera that correlated with the G-type detected in the fecal specimens by multipex PCR. The infecting serotypes detected were G1 (3/9), G2 (3/9), G2, G9 (1/9) and G9 (2/9). Antibody response to the serotypes other than the infecting serotypes was also detected in 7/9 sera by blocking ELISA **(Table 2).**

Ten of the sixteen sera from rotavirus negative children showed absence of anti-rota antibodies. Six sera showed antibody response to G1-G4, or G6, (titers = 1:100) indicating past rotavirus infection. The fecal specimens of these 6 children were negative to all rotavirus serotypes by multiplex G-typing (**Table 3).**

Table 2 : Rotavirus serotype specific antibodies in the sera from rotavirus positive children as detected by blocking ELISA

Sr. No.	Specimen No.	Age/ Sex	Antibody titres against rotavirus serotypes in acute/convalescent sera							G type by PCR (In fecal sp)
			G1	G2	G3	G4	G9	G6 (Bovine)	SA-11 (Simian)	
1	006559	7m/F	-ve/<100	50/100	100/-ve	100/-ve	100/200	100/50	-ve/-ve	G9
2	006583	15m/M	-ve/400	-ve/400	-ve/-ve	-ve/-ve	50/100	-ve/400	-ve/-ve	G2
3	006693	13m/M	-ve/<100	-ve/50	-ve/-ve	-ve/-ve	-ve/50	-ve/50	-ve/-ve	G2, G9
4	006833	1yr/F	-ve/<100	-ve/-ve	-ve/-ve	-ve/50	50/100	-ve/-ve	-ve/-ve	G9
5	006943	6m/Vl	-ve/NA	-ve/NA	-ve/NA	100/NA	100/NA	-ve/NA	-ve/NA	G9
8	022233	5m/Vl	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	G1
7	022235	6m/Vl	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	G1
8	022562	11m/F	ve/<100	ve/ ve	ve/ ve	ve/ ve	ve/ ve	ve/ ve	ve/ ve	G1
9	023257	15m/M	-ve/400	-ve/200	-ve/400	-ve/-ve	-ve/400	-ve/200	-ve/50	G1
10	026237	5m/F	-ve/50	-ve/-ve	-ve/-ve	-ve/-ve	-ve/-ve	-ve/-ve	-ve/-ve	G1
11	026282	15m/F	-ve/50	-ve/200	-ve/-ve	-ve/-ve	-ve/50	-ve/-ve	-ve/-ve	G2
12	026661	18m/M	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	-ve/NA	G2
13	026639	10m/M	ve/NA	ve/NA	ve/NA	ve/NA	ve/NA	ve/NA	ve/NA	G2
14	026736	9m/F	-ve/50	-ve/100	-ve/50	100/-ve	100/-ve	-ve/-ve	-ve/-ve	G2

NA: Not available

Table 3 : Rotavirus serotype specific antibodies in the sera from rotavirus negative children as monitored by blocking ELISA

Sr No	Specimen No.	Age/ Sex	Antibody titres against rotavirus serotypes in acute/convalescent sera							G type by PCR (In fecal sp)
			G1	G2	G3	G4	G9	G6	SA-11	
1	006948	11m/M	ve	ve	ve	ve	ve	ve	ve	ve
2	006950	13m/M	ve	ve	ve	ve	ve	ve	ve	ve
3	02540	9m/M	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	02567	1.5yr/M	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5	02628	2yr/M	50	100	100	100	-ve	-ve	-ve	-ve
6	02647	15m/F	-ve	-ve	100	100	-ve	-ve	-ve	-ve
7	02715	8m/F	100	-ve	100	50	-ve	50	-ve	-ve
8	02824	1yr/F	ve	ve	ve	ve	ve	ve	ve	ve
9	02826	4m/F	ve	ve	ve	ve	ve	ve	ve	ve
10	021321	10m/M	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	021555	14m/F	-ve	-ve	-ve	-ve	-ve	100	-ve	-ve
12	022560	2yr/M	-ve	100	100	100	-ve	-ve	-ve	-ve
13	023221	5m/F	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
14	023223	2yr/M	-ve	-ve	-ve	-ve	-ve	100	-ve	-ve
15	023102	6m/F	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
16	025235	8m/F	ve	ve	ve	ve	ve	ve	ve	ve

Blocking ELISA can be used as a substitute for the neutralization test, which is a tissue culture based ELISA test and requires three days for completion. It can be suitably used with its limitations for the detection of serotype specific neutralizing antibodies in epidemiological studies.

Preparation of egg yolk antibodies against human rotaviruses

Manika Burgohain, Ganesh Dhale, SD Chitambar

Egg is a complete diet for the developing embryo and a supplement for the first few days of life of a chicken. The birds vaccinated against human/poultry pathogens produce eggs having yolks with high level of antibody protein, IgY.

Objectives

- To prepare immunoglobulins against human rotaviruses in egg yolk and evaluate their efficacy by *in vitro* and *in vivo* tests.

Work done

Five month old specific pathogen free (SPF) White Leghorn hens were immunized against commonly circulating human rotavirus (HRV) strains KU(G1P[8]), S2(G2P[4]), YO(G3P[8], ST3(G4P[8]) and F45(G9P[8]). The birds were found to generate anti-rota antibodies, with ELISA titers ranging from 1:50,000 to 1:1,00,000 after third booster dose.

Eggs were collected from the birds and processed for purification of IgY and its protein estimation and anti rota antibody titers. The protein content of the purified IgY was in the range of 2.4-3.5 mg/ml and antibody titers varied from 1:64,000 to 1:256,000 in various lots.

Purified IgY antibodies from different lots of immnune eggs were tested for its neutralizing activity against homologous and heterologous virus strains by cell culture based neutralization assay. Neutralization titers were observed to be higher against homologous viruses (=1: 6400) while the same were lower against heterologous viruses (=1: 800) (**Table-4).**

Table 4 : Neutralizing antibody titers against rotaviruses

IgY	Neutralizing antibody titers				
	HRV-1	HRV-2	HRV-3	HRV-4	HRV-9
IgY HRV-1	>6400	200	800	200	400
IgY HRV-2	400	6400	400	200	400
IgY HRV-3	200	200	>6400	400	400
IgY HRV-4	400	200	200	>6400	400
IgY HRV-9	800	200	400	800	6400

To test *in-vivo* efficacy of the antirota IgY antibodies, an infant mouse model was developed. Four to five days old BALB/c infant mice were inoculated orally with HRV-3(YO) and a single diarrheal dose of 50 µl having 1:2000-1:4000 EEP infectious titer was able to produce diarrhea in 70-90% of animals expressing loose yellow faeces. Maximum numbers of animals were observed to be diarrheic at 48 hours of post infection.

Hospital based surveillance of non-rota enteric viruses in acute gastroenteritis patients

SD Chitambar and V. Gopalkrishna

Acute gastroenteritis is one of the most common diseases in humans and continues to be a significant cause of morbidity and mortality worldwide. Among enteric viruses, rotavirus is the leading viral agent associated with severe diarrhea especially in infants and young children. However, some patients develop diarrhea with non-rotavirus infections indicating involvement of other enteric viral or bacterial pathogens. Recently, association of other enteric viruses such as Calici, Astro, Adeno, Entero viruses have been reported in sporadic and outbreak cases of diarrhea in Asian and European countries and US. In India, limited studies are reported on other enteric viral pathogens. The causative agents in such cases are rendered unidentified in the absence of concerted efforts in most of the episodes of gastroenteritis. It is essential to study the spectrum of unknown viruses in sporadic and outbreak cases of gastroenteritis.

Objectives

- To determine the proportion of diarrhea cases attributable to Calici, Astro, Adeno and Entero viruses in sporadic infections and outbreaks of gastroenteritis in India.

Identification and molecular characterization of Norovirus strains in acute gastroenteritis patients from western India

Preeti Chhabra and SD Chitambar

A total of 830 fecal specimens were collected from acute gastroenteritis patients aged = 7 years, admitted to the hospitals or visiting OPDs in different cities of western India during July 2005 - June 2007. These included 570 specimens (520 IPD and 50 OPD) from Pune, 70 (49 IPD and 21 OPD) from Aurangabad and 190 specimens (IPD) from Nagpur. All specimens were tested for norovirus RNA by RT-PCR using RNA polymerase region primers specific to genogroup I and II.

Of 570 specimens collected from Pune, noroviruses were detected in 72 specimens (12.8%), which included 66/530 (12.4%) IPD and 6/50 (12%) OPD patients. While 70/72 (97.2%) specimens showed positivity for GII noroviruses, 1/72 (1.3%) was positive for GI and 1/72 (1.3%) specimens showed mixed infection with both GI and GII noroviruses. In comparison, 5/70 (7.1%) specimens from Aurangabad were found positive for GII noroviruses including 4/49 (8.1%) IPD and 1/21(4.7%) OPD cases. Twelve of 190 (6.3%) specimens from Nagpur showed norovirus positivity that included 10 (83.3%) GII infections while 2 (16.6%) of GI. Overall, in western India norovirus positivity varied from 6.3 to12.8% with GII being predominant genogroup (98%).

Analysis of clinical severity scores indicated very severe (4.8%), severe (57.3%), moderate (35.3%) and mild (2.4%) disease in hospitalized patients. In comparison to this, 28.5% experienced severe, 57.1% moderate and 14.2% mild disease in outpatients. Nearly 32% and 43% patients did not experience vomiting in hospitalized and OPD cases, respectively.

Norovirus positivity rates were found to be high in children ≤ 2 years (0-36 months) of age (85.7%). Positivity rate decreased in children with higher age groups and were lowest in children 6-7 years of age **(Fig. 2).**

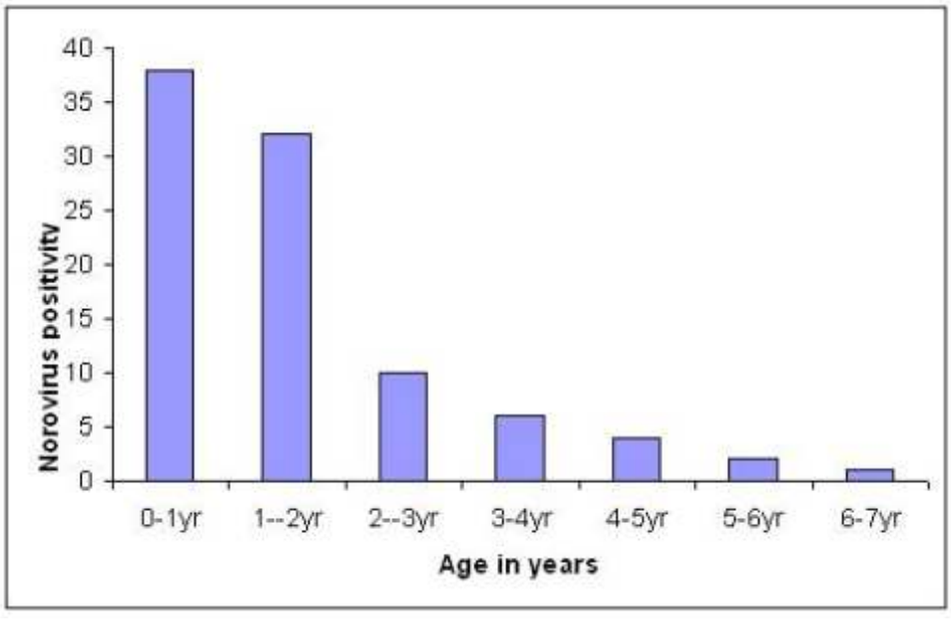

Fig. 2 : Age dependent distribution of norovirus infections in sporadic cases of acute gastroenteritis

Norovirus infections were observed throughout the year. However, norovirus positivity gradually increased in January and was highest in March in both the years 2005-07. Summer month seasonality supported norovirus infections in western India **(Fig. 3)**

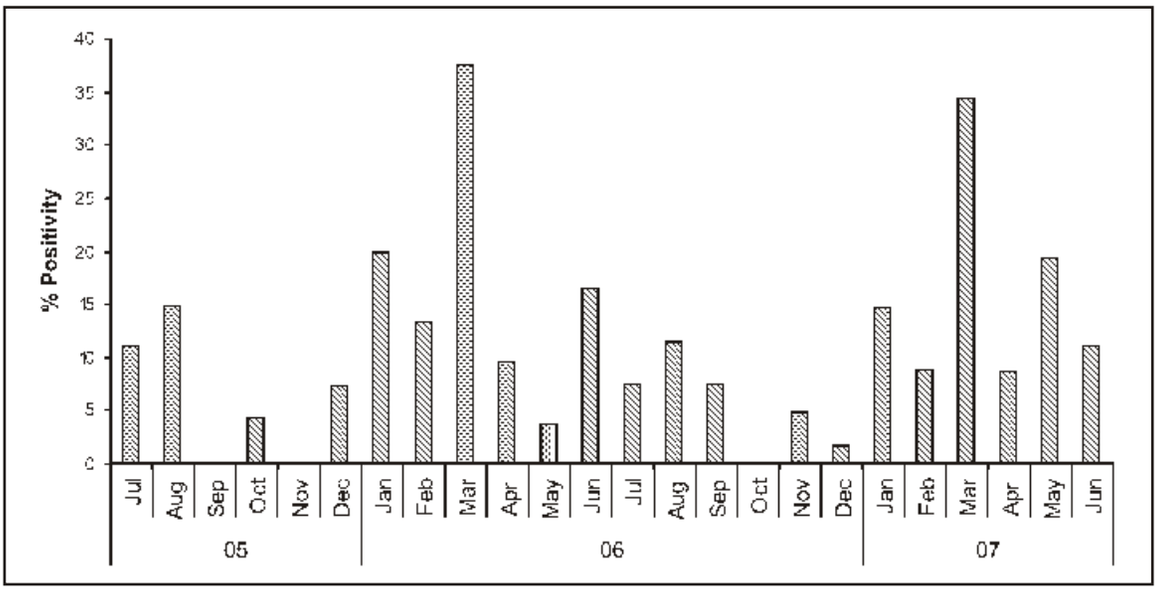

Fig. 3 : Monthwise positivity of norovirus infections in western India

Hospital based detection and characterization of enteric adenovirus and astro virus strains in acute gastroenteritis patients

Harsha Verma, SD Chitambar and V. Gopalkrishna

A total of 228 fecal samples were collected from June 2006 to August 2007 from children with acute gastroenteritis hospitalized at Govt. Medical College, Nagpur, India. Fifty-seven samples were also received from local hospitals of Pune, in January - December 2007. All samples were tested for the presence of adenovirus by PCR using Hexon gene primers. Adenovirus DNA was detected in 7.5% of Nagpur samples and 12.3% of Pune samples respectively. Sequencing of PCR products showed presence of serotype 31 in 10, serotype 40 in 6 and serotype 41 in one of the specimens from Nagpur. The serotype 41 was predominantly detected in Pune samples.

Fecal samples collected from Aurangabad (n=100, May 2005-Feburary 2006), Nagpur (n=228, June 2006-August 2007) and Pune (n=131, January-December 2007) were tested for the presence of astrovirus by RT-PCR using ORF 1a gene primers. Total astrovirus prevalence in Aurangabad, Nagpur and Pune was found to be 4%, 3.5% and 2.3% respectively. Sequencing of PCR products revealed predominance of astrovirus serotype 8 in these regions.

Hospital based detection and characterization of enterovirus strains in acute gastroenteritis patients

PR Patil, SD Chitambar and V. Gopalkrishna

A total of one hundred and eighty fecal specimens were collected from sporadic acute diarrhea cases, admitted at Govt Medical College, Aurangabad (n=95, May 2005-February 2006) and Govt. Medical College, Nagpur (n=85, June 2006-Feb 2007). All samples were tested for the presence of enterovirus RNA by RT-PCR using 5' non-coding region primers. Nearly, 22 and 8.9% of the fecal specimens showed the presence of enterovirus RNA from Aurangabad and Nagpur respectively. Sequencing and phylogenetic analysis showed distribution of enterovirus as- CA-1(n=3),CA-19(n=1),CA-22(n=1),CA-20(n=1),CA-10(n=7),CB-2(n=4),EV-90\91(n=1),EV-89\76(n=1),HEV-B(n=2) at 90-98% level in Aurangabad and EV-89\76(n=3), EV-90\91(n=1),CA-1\CA-22 (n=2) at 94-98% level in Nagpur. In addition, the 119 fecal samples collected from acute diarrhea cases from Pune hospitals during 2006

were also tested for enterovirus. Fifteen (12.6%) samples were found to be positive for EV-RNA by RT-PCR.

Additional Studies

Investigation of Outbreaks of Acute Hemorrhagic Conjunctivitis (AHC) occurred in Maharashtra and Karnataka states during 2006 and 2007

V. Gopalkrishna, PR Patil and SD Chitambar

An outbreak of acute hemorrhagic conjunctivitis was reported during October-November 2006 in Pune, India. Thirty eye swabs collected from the patients were subjected to virus isolation in HeLa cell line. Enterovirus and adenovirus detection was carried out by RT-PCR (5'NCR) and DNA PCR (Hexon region) respectively. Fourteen of the 30 cultures inoculated with clinical specimens showed CPE after blind passaging. All 14 isolates were found positive for enterovirus RNA by RT-PCR. Sequencing of PCR products showed 98-99% nucleotide homology with DSO isolate of Coxsackie A24 from Singapore. Further, eleven of the 14 isolates showed amplification in the VP3-VP1 region using CA-24 specific primers and revealed 96.9-98.0% homology with Singapore strain, 95.8-97.4% with China strain and 94.9-96.7 % with Spanish strain. PNI within isolates was observed to be 98.9-100%. None of the isolates showed presence of adenovirus.

Similar AHC outbreaks were also reported from Pune, Mumbai, Nagpur (Maharashtra) and Banglore (Karnataka) cities in October-November 2007. A total of 60 eye swabs collected from conjunctivitis patients from Banglore (25), Pune (10), Mumbai (10) and Nagpur (15) were tested for enterovirus and adenovirus. Out of sixty clinical samples analyzed, twenty-two (36.6%) were found to be positive for enterovirus by RT-PCR. None of the clinical samples showed the presence of EV70 and adenovirus. Sequencing and phylogenetic analysis of the 5'NCR of enterovirus showed close homology with DSO isolate of CA-24 strains from Singapore.

Publications

- Gopalkrishna V, Patil PR, Kolhapure RM, Bilaiya H, Fulmali P, Deolankar RP. An Outbreak of Acute Hemorrhagic Conjunctivitis caused by Coxsackie-A 24 variant in Gujarat and Maharashtra states in India**.** **J Med Virol** 2007: 79(6): 748-753.
- Gadgil PS, Fadnis RS, Joshi MS, Rao PS, Chitambar SD. Seroepidemiology of hepatitis A in Voluntary blood donors from Pune, Western India 2002 and 2004-05. **Epidemiol Infect.** 2008 Mar; 136(3): 406-9.

Workshops / Conferences / Seminar / Meetings attended

SD Chitambar

- Investigators meeting of the Multicentric project on Hospital Based Rotavirus Surveillance in India held at EVRC, Mumbai, on 14th May, 2007.
- Task force meeting on Hospital Based Surveillance Network for Rotavirus Disease and Strains organized by ICMR at Mumbai on 17th September, 2007
- Workshop on Norovirus detection and typing organized by ICMR and CDC, Atlanta, USA at ERC, Mumbai on 18th-21st Sept. 2007
- Invited lecture on “Rotavirus viremia in acute diarrhea patients: Variance in strains detected in stools and sera” at International Conference on Emerging & Re-Emerging Viral Diseases of the Tropics & Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

- Task force meeting on Genomics and Molecular Medicine for presentation on the project entitled Genomic analysis of Hepatitis A virus isolates from different geographic locations of India, at ICMR New Delhi on 1st February 2008.
- Advanced training in Characterization of untypeable rotavirus strains at CDC, Atlanta from 20th February 2008 3rd April 2008.

V Gopalkrishna

- Molecular characterization of an etiological agent that caused outbreaks of Acute Hemorrhagic Conjunctivitis in Maharashtra and Karnataka states of India, 2006-2007. International conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

VS Tatte

- Uncommon and Mixed Rotavirus infections in Adolescent and Adult cases of acute gastroenteritis from Pune, western India". 5th World Congress of The World Society For Pediatric Infectious Diseases, held in Bangkok, Thailand between 15th-18th November 2007.

Ritu Arora

- Workshop on Norovirus detection and typing organized by ICMR and CDC, Atlanta, USA at ERC, Mumbai on 18th-21st Sept. 2007.
- Sequence analysis of VP4 and VP6 genes of human rotavirus G1P [8] after serial passages in MA104 cells. International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

Preeti Chhabra

- Workshop on Norovirus detection and typing organized by ICMR and CDC, Atlanta, USA at ERC, Mumbai on 18th-21st Sept. 2007.
- Contribution of noroviruses in causing acute gastroenteritis in Pune, Western India. International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

MM Yadav

- Workshop on "Knowledge Discovery in Life Sciences: Modeling Small Molecules to Macromolecules". Organized by Department of Bioinformatics, University of Pune from 11 17th Dec. 2007.

Harsha Verma

- Detection and molecular characterization of enteric adenoviruses among infants and children hospitalized for acute gastroenteritis. International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

Shilpa Bhalla

- Genomic Analysis of Hepatitis A virus isolates from different geographic locations of India“ by, International Conference on Emerging and Re-emerging Viral Diseases of the Tropics and Sub-Tropics, organised by Indian Virology Society at Indian Agricultural Research Institute, New Delhi during December 11-14, 2007.

Patent filed

A patent is filed on the “Use of IgY antibodies against rotavirus infection in children and poultry” jointly by National Institute of Virology and Venkeys (India) Limited.

Measles

Measles

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10 Measles

- Measles Aerosol Vaccine Trial
- Molecular Surveillance of Measles in India
- Isolation and Genotyping fo Measles viruses from specimens referred by WHO - SEARO, measles laboratories
- Molecular Epidemiology of Measles in North-East India
- Measles M gene sequencing based diagnosis of subacute Sclerosing PanEncephalitis (SSPE)

Measles Aerosol Vaccine Trial

Collaborators : King Edward Memorial (KEM) Hospital and National AIDS Research Institute (NARI), Pune

NS Wairagkar, NJ Shaikh, LV Hungund, NS Kumbhar, RS Tomar

Measles group is involved in Phase I Measles aerosol vaccine trial since 2006 and the project continued in this year. Group 1 (18-35 years) was followed up to their 12th visit of the subjects in June 2007. Total of 19 subjects completed the follow-up for one year. The data was sent to WHO and preparation continued for screening and recruitment of Group 2 (5-17 years).

Data Safety monitoring Board DSMB gave approval to go ahead with Group 2 (5-17 years) screening. Accordingly, 119 subjects of this group were screened for clinical, hematological and biochemical criteria and 20 subjects were chosen for inclusion in the study. These were vaccinated with aerosol route and were followed up with regular visits on day 1,3,7,10,14, 28 and 90. Acute toxicity and safety data was assessed on 14 and 28 day. Data was submitted to DSMB, which permitted us to go ahead with Group 3 (1-4 years) in the trial. WHO Product Development Group (PDG) meeting was organized in Pune, which reviewed the trial findings and initiated preparations for next phase of the trial.

DSMB gave approval to go ahead with Group 3 (1-4 years) screening. Accordingly, 64 subjects of this group were screened for clinical, hematological and biochemical criteria. 15 subjects were found eligible on all the criteria and were vaccinated with measles vaccine by aerosol route. The subjects were closely monitored till day 28 as post vaccination follow-up. The group will be monitored for the safety aspects for one year till visit 12.

PRNT support was extended to all the three sites Pune, Kolkata and Chennai. Over 400 specimens from Screening, day 28 and day 90 post vaccination were screened with PRNT for measles antibodies.

GCLP external audit

GCP and GCLP compliance is the basic requirement for participation in International Vaccine trials. Since NIV measles laboratory is a part of Measles Aerosol Vaccine trial, attempts were made to improve the compliance. Various steps taken for GCLP compliance include, Implementation of EQAS program with Health Protection Agency; establishment of Internal Quality Assurance Unit at NIV and implementing IQA SOPs including, internal panel creation and testing, SOP based, protocol based and facility based internal audit; Development of departmental manual with study plan and SOPs required for the vaccine trial; preparation of Quality Manual for PRNT; designing and implementation of Equipment Calibration Program in the department. All these programs were undertaken in the reporting year, staffs were trained internally and at two external GLP workshops. This was the major work done initiating the whole process of GCLP compliance in NIV labs.

External QMS Professionals judged GCP and GCLP compliance this year. They conducted 2 external audits of the Measles lab. After the first audit, Corrective Action Plan (CAP) was prepared for the Scientific Audit Report and preparation for compliance to GLP continued. In the final scrutiny, Audit observed a huge improvement in the lab as compared to first audit and all the critical and major issues were addressed with due care and adherence to the ICH guidelines. **NIV's Measles lab** is now **declared as GCLP compliant lab for vaccine trial** leading to honor of being the first **GCLP compliant ICMR laboratory.**

Molecular Surveillance of Measles in India

Collaborators : *MeaslesNetIndia*

NS Wairagkar, RS Tomar, LV Hungund, NJ Shaikh

Measles group has completed measles virus genotyping project, funded by WHO-IVR. Measles genotypes circulating in 17 states of India were sequenced. The Measles Sequence Database is created at NIV and has over 200 sequences. This database is growing day by day. *MeaslesNetIndia* (NIV-ICMR research network for Measles and SSPE) established for the project is expanded to 27 centers after addition of 10 centers from North-East region India.

Measles outbreaks in Jammu and Kashmir, Agartala, Orissa, West Bengal and Madhya Pradesh were investigated in the current year of reporting. RT-PCR and sequencing was done and genotypes of measles circulating in these states were detected **(Fig. 1).**

The project period was over in August 2007**.** During the total period of two years, we have investigated 32 outbreaks, detected around 172 strains with genotypes belonging to D4, D7 and D8**.** Genotype D7 is detected for the first time in India. The interim project report is submitted to WHO-IVR for review. The comments on the report are awaited.

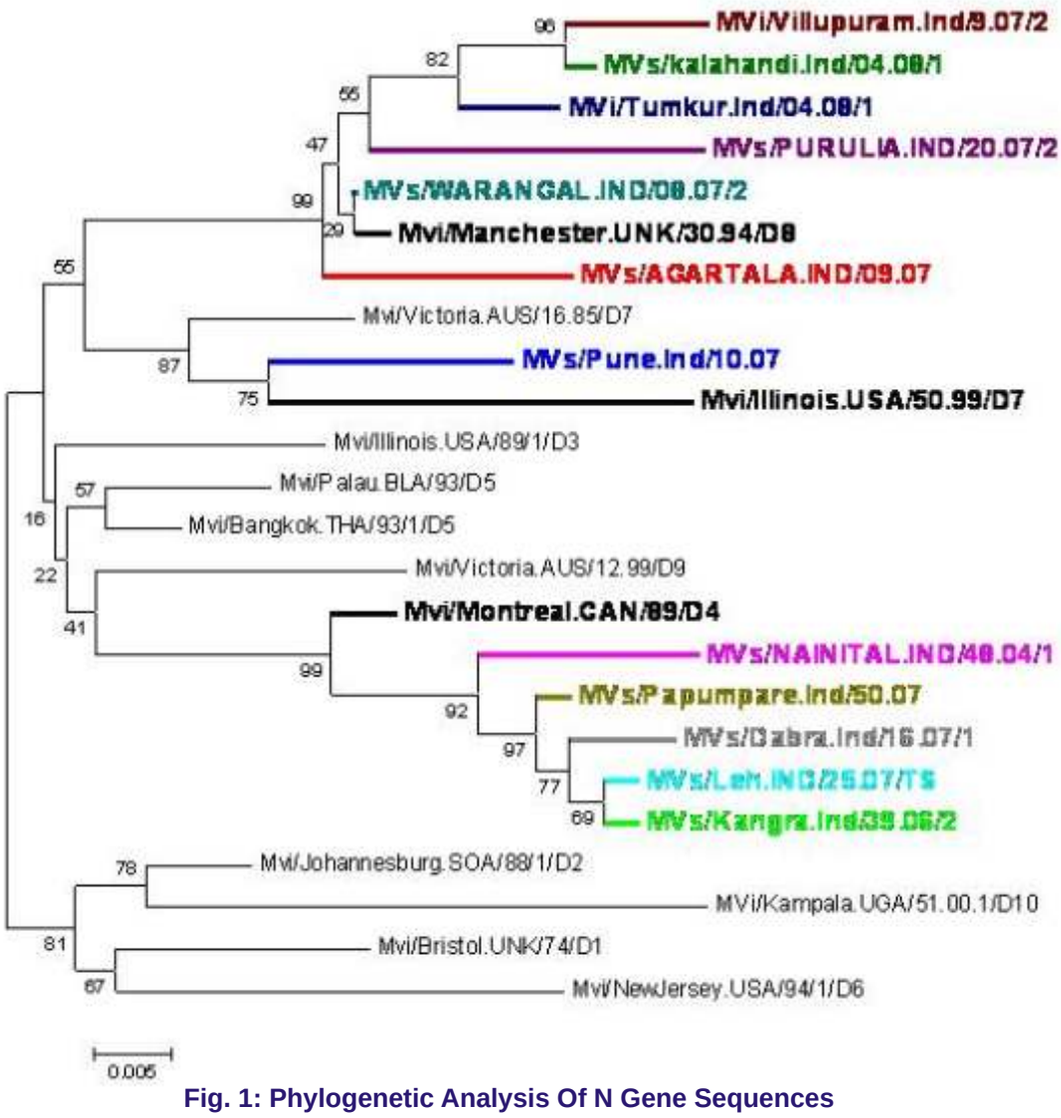

Fig. 1: Phylogenetic Analysis Of N Gene Sequences

Jammu & Kashmir State

Collaborators : Dr. SM Kadri, Directorate of Health Services, Kashmir, Dr. S Slathia, SMGS Hospital, Govt. Medical College, Jammu

Three outbreaks of measles were investigated in J & K in the district of Leh, Kargil and Jammu in 2007. Age group ranged from 6 months to 28 years. Below 12 months only female children were affected. From 13 months to 156 months more number of males were affected than females.

Leh Outbreak: A total of 355 cases of measles were detected in Leh district. No complications or casualty was found. The immunization coverage in J & K is around 85%. March second and third week was the peak of outbreak. Three serum samples were sent to NIV. All cases were from adolescent age (all 13 yrs). Serologically the outbreak was confirmed to be measles. T.S & / or urine were not available for RT-PCR.

Kargil Outbreak: Measles outbreak occurred in Kargil district (population of 1,07,138) from 1.05.07 25.06.07. A total of 38 cases (6mths to 28 yrs) were detected. Representative samples were collected from four cases. First case in 6 months old female of whom T.S was positive for measles by RT-PCR. Second case (1 yr old, Female) has received measles vaccine was also positive by RT-PCR. Third case (2 yrs male) was vaccinated for measles and showed symptoms only fever & rash. IgM ELISA was positive. Fourth case was 28 yrs pregnant female in her last trimester. She presented with fever, rash, cough & cold. Measles IgM antibodies were detected. She delivered a normal baby with no complications. Measles genotype D4 detected in all these cases.

Cluster from Jammu: Sporadic cases of measles were admitted in Government Medical College, Jammu from Nov. 2006 to Feb. 2007. All 5 cases were admitted with history of fever, cough, rash and lymphadenopathy. IgM ELISA was positive in 1/5 sera collected. Throat swabs of these cases were PCR positive and D4 genotype was detected from all four cases.

Tripura State

Collaborators : Dr.S Debbarma, Agartala Govt. Medical College, Tripura

Agartala Outbreak: MeaslesNetIndia collaborator from Agartala, Tripura, investigated small cluster of 6 measles like cases in the month of March and April 2007. Four cases were from Agartala and two cases were from a locality 15-18 kms from Agartala. No complications were found. Age group ranged from 8 months to 17 years. Male to female ratio was 4:2. Most of the cases (4/6) were unvaccinated. Maximum cases occurred in the age group of 3-6 years. A total of 24 samples were sent, out of which 6 were serum, 6 urine, 6-throat swab (TS) and 5 oral swab (OS) samples. Serological study showed IgM antibodies to measles in only one serum sample and also RT-PCR positive result from throat swab and oral fluid sample of the same case. Rest of the serum samples, TS and oral fluid samples were negative. This measles confirmed case was 17 years old and not vaccinated for measles. The genotype detected was D8.

West Bengal State

Collaborators : Dr. AK Biswas, Deputy Chief Medical Officer of Health, Purulia, Dr. Bishnupada Bag, Assistant Chief Medical Officer of Health, Murshidabad and Dr. Shobhan De , FETP Scholar, Purulia

Purulia Outbreak : An outbreak of measles occurred in Kantadin, Jhalda villages of Punulia WB in February 2007. Age group of case ranged from 3 and half years to 7 years. M:F ratio is 3:4. Only one case was vaccinated. 7 serums samples, 7 T.S and 7-urine samples were received. IgM ELISA was positive for 6/7 serum samples. RT-PCR is under process.

Neturia outbreak : block was investigated in May 2007, where serum, urine and TS were collected from five cases each. Age ranged from 3.5-10 yrs of age (M: F ratio 3:2). None of the cases were vaccinated. Five serum samples were tested for IgM ELISA and all five were positive. 3/5 TS were positive for RT-PCR. The genotype detected is D8 in all the 3 cases.

Murshidabad outbreak : Measles outbreak occurred in Dihigram village, Suti-II block of Murshidabad in the month of May 2007. 11 cases were reported from this village with a population of 1750 and less than 10 years population of 350. Serum and urine samples were received from 11 cases. Age group ranged from 8 months-7 yrs. All were unvaccinated. 9/11 serum samples were positive for measles IgM ELISA. All urine samples were negative by RT-PCR.

Madhya Pradesh State

Collaborators : Dr.Ketkar, Ketkar Hospital, Gwalior

A small cluster of five measles cases was investigated at Dabra in Madhya Pradesh in the month of March 2007. Age group ranged from 6 months to 6 years. More number of males (4/5) were affected than females. Only one case was vaccinated for measles. All 5 sera were positive by IgM ELISA. Three oral fluids were positive by RT-PCR and all were D4 genotype. (Annexure- phylogenetic tree from N gene sequences)

Orissa State

Collaborators : Dr. Tapas Kumar Patra, Chief District Medical Officer, Kalahandi

Cluster of 8 measles cases in Kalahandi, tribal district of Orissa was investigated with the help of public health officials. Age group of cases ranged from 4 to 8 years. M:F ratio is 5:3. History of measles vaccination was unknown in all the cases. Eight serums samples and 6-urine samples were received from eight patients. Measles IgM ELISA was positive for 7/7 serum samples. RT-PCR for measles N gene was positive for 2/6 urine samples. Both the sequences belong to measles D8 genotype. Kalahandi is tribal district in Orissa state where measles related malnutrition is a major public health problem.

Isolation and Genotyping fo Measles viruses from specimens referred by WHO - SEARO, measles laboratories

NS Wairagkar, NS Kumbhar, Deepika Khedekar

In this year, **WHO SEARO recognized Measles lab of NIV, Pune as the Reference Measles Laboratory** with the assigned task of virus isolation and measles genotyping for WHO Measles Laboratory Network. As a part of this assignment, 13 isolates were referred from National Measles Laboratory, Chennai and 4 isolates were referred from National Measles Laboratory, Banglore. All the isolates were confirmed as measles at NIV and were sequenced for N and H gene. Measles genotypes D8 (12 isolates), D4 (1 isolate) were detected in Chennai and D8 (four isolates) in Bangalore. Sequences were deposited in WHO's Global Measles Sequence database. This database will be used to track the transmission pathways of measles strains.

Molecular Epidemiology of Measles in North-East India

Collaborators : Regional Medical Research Center, Dibrugarh

NS Wairagkar, RS Tomar, LV Hungund.

In collaboration with RMRC, Dibrugarh, a new project on Molecular Epidemiology of Measles in North-east India was initiated this year with funding from the ICMR's NE Initiative. The objective of this project is to create, develop and strengthen ICMR's Measles Network in Northeastern states. The strategy would be to investigate the measles outbreaks, confirm serologically and try isolations of measles strains circulating in the region and conduct molecular studies including sequencing for understanding the molecular epidemiology of measles in NE region. The WHO-India-funded workshop was organized at Dibrugarh to train the collaborators from ten centers from NE Region on methodology for measles outbreak investigations, collection of specimens, serological studies etc. 23 collaborators attended the workshop from 10 centers initiating the MeasleNetIndia-NE network. The staff of project based at RMRC Dibrugarh was trained in outbreak investigations, collection of specimens, serological and virus isolation techniques. Attempt will be made to strengthen the virology lab at RMRC for measles diagnostics.

Measles M gene sequencing based diagnosis of subacute Sclerosing PanEncephalitis (SSPE)

Collaboraters : Dr. SK Shankar, NIMHANS, Banglore

NS Wairagkar, Deepika Khedekar

Subacute sclerosing panencephalitis (SSPE), a post-measles progressive neurological disorder is still common in India because of indifferent vaccination compliance. However, the acute fulminant form of SSPE is extremely rare. An unusual case of fulminant SSPE in an 18 year old man from south India with an ultra-short course of 19 days presenting with hemiparesis in absence of myoclonus and progressive cognitive decline, is reported. At autopsy, unlike classical SSPE, oligodendroglia containing measles viral antigen was sparse despite florid necrotizing leukoencephalitis with acute demyelination. Since histopathology alone could not finalize the diagnosis in this case, molecular methods were used.

Measles M gene sequencing is important to confirm the diagnosis of SSPE. PCR and Sequencing M gene protocols were standardized on RNA extracted from brain tissue sample. Sequence of 1008 bp was obtained from position 3438nt to 4445nt of measles genome. Bang.Ind-SSPE strain showed maximum similarity (92%) to

measles virus isolate MVs/Dundee.UNK/82/SSPE (DQ190374) (**Fig -2**).

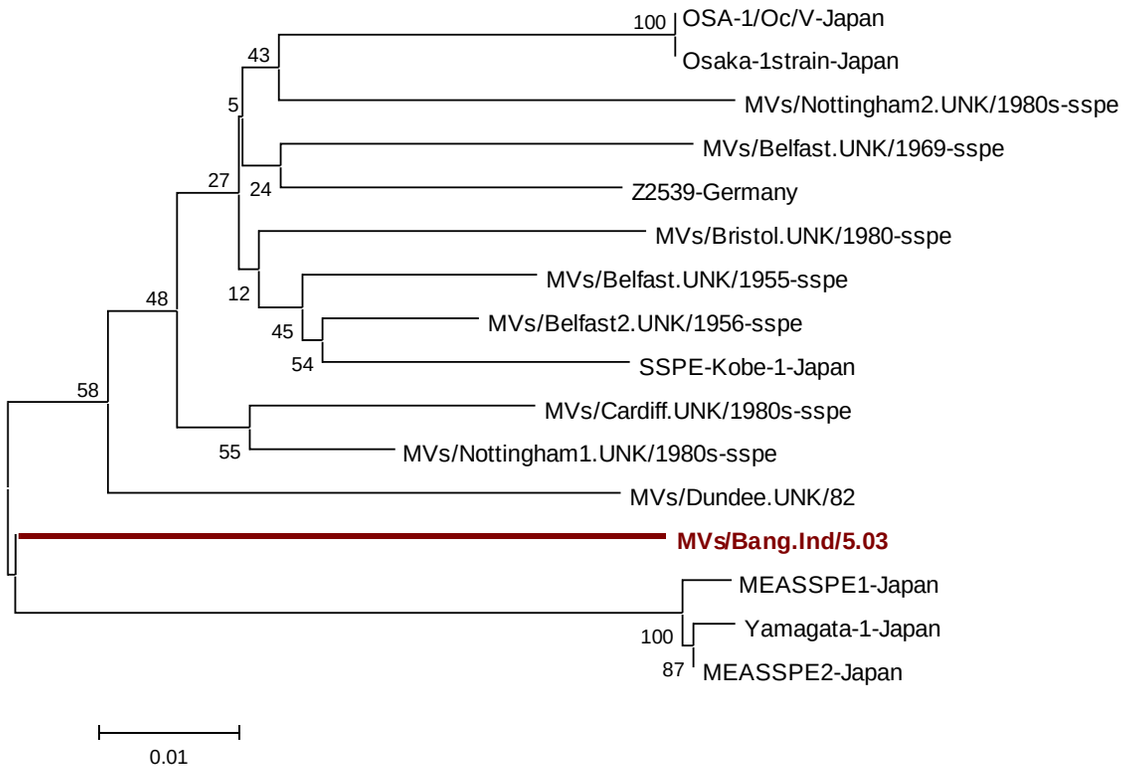

Fig. 2 : Phylogenetic analysis of Measles Matrix gene sequences

Biased Hypermutations in the matrix gene (mainly U to C) have been known as characteristic of SSPE. We compared M gene nucleotide sequence of Bang.Ind-SSPE strain to that of D7 measles virus isolate from Boston, MA 1983 strain, (Bo83/US/83 Genbank accession No. U01985), and M gene sequence of Edmonston strain (Genbank accession No.K01711). A considerable amount of mutations were found along the entire length of M gene. Compared with Boston strain there were mutations at 91 sites along the M gene-coding region, majority (80.2%) of the mutations were U-to-C transitions. Similarly compared to Edmonston strain, 80.9% were changes from U to C.

Our strain was compared with 110 M gene nucleotide sequences (SSPE and classical measles) available in GenBank and NCBI database. Five unique mutations in our SSPE strain were noted as Adenine substituting Guanine or Cytosine (at position 170/1008 M gene) and Cytosine substituting Thymine (at positions 600/1008, 614/1008, 780/1008, and 981/1008). Comparison from deduced amino acid sequences showed three unique mutations All these findings confirmed our diagnosis of SSPE and have pointed out some unique mutations in our SSPE strain, which were not reported before. Standardisation of M gene sequencing will pave way for more studies on SSPE in India.

Publications

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- Cohen BJ, Audet S, Andrews N, Beeler J; WHO working group on measles plaque reduction neutralization test. (E. Arias Toledo, S. Audet, J. Beeler, W. Bellini, J. Bennett, M. Bentley, D.W.G. Brown, D. Doblas, D. Featherstone, A. Heath, A.M. Henao Restrepo, R. Parry, J. Ruiz Gomez, N. Shaikh, N. Wairagkar). Plaque reduction neutralization test for measles antibodies: Description of a standardised laboratory method for use in immunogenicity studies of aerosol vaccination. **Vaccine**, 2007; 26/1:59-66.
- Sapkal GN, Wairagkar NS, Ayachit VM, Bondre VP, Gore MM. D e t e c a t n i o b s o l a t f J i a o p n a n e s e e n c e p h v a i l r f u r s o b s l o o b l o c t o s l l e d u t r e a d k t g u p t h e a s e f i n f e **Am J Trop. Med Hyg.** 2007 Dec;77(6):1139-45

Workshops / Conferences / Seminar / Meetings attended

NS Wairagkar

- Residential conference of Measles Laboratory Coordinators, Udaipur, 3 - 5 March 2008.
- WHO meeting of 9th Product Development Group of Measles Aerosol Vaccine Project, Geneva, 28-31 January 2008.
- WHO meeting of 8th Product Development Group of Measles Aerosol Vaccine Project, Pune, 31st October-2nd November 2007.
- WHO fifth Global Measles and Rubella Laboratory network meeting, Geneva, 26-28 September 2007.
- WHO-Ministry of Health - India Technical Advisory Group on Measles Control, New Delhi, September 2007.
- Asia Pacific Dengue Prevention Board Meeting, Colombo, Sri Lanka, 20-24 June 2007.
- WHO-NIV workshop on MeaslesNetIndia- molecular surveillance network for measles in NE India, at RMRC, Dibrugarh, 23-24 July 2007.
- Eight Advanced Vaccinology Course, Fondation Marieux, Annecy, France, 14-28 May 2007.
- WHO Meeting on Measles Aerosol Project Review, Mumbai, 10th May 2007.

LV Hungund

- WHO-NIV workshop on MeaslesNetIndia- molecular surveillance network for measles in NE India, at RMRC, Dibrugarh, 23-24 July 2007.
- IBSC workshop on Biomedical Waster Management, Pune 29 Oct, 2007.

NJ Shaikh

- UNDP/WHO/World Bank workshop on Good Laboratory Practice. Bangalore, 29-31st Oct 2007.
- WHO Meeting on Measles Aerosol Project review, Mumbai, 10 May 2007.

VS Bhide

- UNDP/WHO/World Bank workshop on Good Laboratory Practice, Bangalore 29-31 Oct, 2007.

RS Tomar

- IBSC workshop on Biomedical Waster Management, Pune, 29 Oct, 2007,.

Trainings/Workshops/Seminars organized

WHO-NIV workshop on MeaslesNetIndia-molecular surveillance network for measles in NE India, at RMRC, Dibrugarh, 23-24th July 2007.

Entomology

Entomology

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11 Entomology

- Geographic genetic variation among natural populations of *Culex tritaeniorhynchus* and susceptibility to infection with JE virus
- Entomological studies in the arbovirus affected areas

Geographic genetic variation among natural populations of *Culex tritaeniorhynchus* and susceptibility to infection with JE virus

PC Kanojia and MD Gokhale

Introduction

Four populations of *Culex tritaeniorhynchus* collected from Bellary, Cuddalore, Pashan and a laboratory strain were colonized and studied for morphological and allozyme variations. Among these one population of *Culex tritaeniorhynchus* (Bellary) has shown endophilic behavior in nature. The proposed study was planned to understand this behavioral feature on the basis of comparative morphological and allozyme patterns with reference to exophilic strains of *Culex tritaeniorhynchus* viz. Cuddalore and Pashan strain.

Objective

- To compare the different geographic strains of *Culex tritaeniorhynchus* on the basis of morphometric analysis.
- To compare the different geographic strains of *Culex tritaeniorhynchus* on the basis of allozyme analysis.

Work don

- Different geographic strains of *Culex tritaeniorhynchus* were collected and colonized.
- Morphometric measurements of 7 important taxonomic characters were carried out
- Isoenzyme parameters of these strains were studied using PolyAcrylmideGel Electrophoresis (PAGE)
- The data generated on morphological and isoenzyme measurements was subjected to the statistical analysis

Summary

Principal component analysis of the data suggest that among eight morphological characters, three characters viz. siphon, saddle and anal gills were most important.

Discriminate factor analysis of morphological data suggests that the four populations form significantly different clusters.

The allelic frequencies and Nei's genetic identity showed that genetic differences between populations were small but significant.

Morphological and allozyme variations in the *Culex tritaeniorhynchus* populations could be attributed to the environmental conditions. It is suggested that transition of morphological characters and allozyme variations in *Culex tritaeniorhynchus* populations is a consequence of environmental conditions selection.

Future Plan

It is proposed to study the Japanese Encephalitis (JE) virus susceptibility status among these populations of *Culex tritaeniorhynchus*.

Entomological studies in the arbovirus affected areas

MD Gokhale

Introduction

Entomological investigations with reference to vector prevalence and virus isolations were conducted at different

arbovirus affected areas viz. Satara, Kolhapur, Jabalpur, Kerala, Barsi and Pune Urban Agglomeration.

Objectives

- To asses the prevalence of suspected vector species in the affected areas.
- To attempt the isolation of causative viral agent of the disease among the field caught vector specimens.

Work done

- Entomological studies were carried out at Jabalpur (M.P.) on two consecutive visits in the month of September. Mosquito vector survey in relation to Dengue virus outbreak was conducted in the affected areas. *Aedes aegypti* larval and adult indices were comparatively high during both the visits despite anti larval measures by the local authorities **(Table 1).**
- Two districts of western Maharashtra viz. Satara and Kolhapur reported Chikungunya virus activity in the month of April and May. *Aedes aegypti* presence was detected in all the affected areas **(Table 2).**
- Field surveys were conducted in two Chikungunya virus affected districts of Kerala viz. Mallapuram and Kozikodee in the month of October. In all, a total of 9 localities were visited. Following *Aedes* species were encountered in and around the affected areas viz. *Aedes albopictus*, *Aedes aegypti* and *Aedes vittatus*. Among these *Aedes albopictus* was the predominant species in and around the affected households; while *Aedes aegypti* was represented only in the automobile tyres on the state highways. A colony of *Aedes albopictus* and *Aedes aegypti* mosquitoes from Kerala has been established in the laboratory for further studies **(Table 3).**
- Entomological studies with reference to Chandipura virus activity were conducted at Barsi (Maharshtra) in the mouth of October. A total of 4 affected localities were surveyed and 303 sandfly individuals belonging to *Phlebotomus* and *Sergentomyia* genera were collected **(Table 4).** The sandflies were processed for virus isolation. No virus agent was detected.
- Studies were conducted with reference to Chandipura virus activity in six districts of Vidarbha region of Maharashtra. Sandfly species were collected in domestic and peri-domestic sites in the affected areas. The details of the sandflies collected are given in **Table 5.**
- In response to the sudden increase in Dengue cases in the PMC and PCMC areas as evident by IgM positivity of the serum samples, *Aedes aegypti* surveys were conducted in the affected households with reference to the density of the vector species and the detection of the virus activity in the field caught adult mosquitoes. A total of 20 localities comprising of 400 houses were visited during the survey. The prevalence of *Aedes aegypti* in the PMC and PCMC was more or less similar **(Table 6).** A total of 31 *Aedes aegypti* adults were collected. All were negative for the presence of Dengue virus **(Table 7).**

Table 1: Entomological studies during Dengue outbreak at Jabalpur

Sr. No. of visits	No. of localities searched	No. of Man Hours spend	No. of houses searched	BI	PI (%)	AHI (%)
1	4	8.0	170	14.7	11.1	6.4
2	8	16.0	210	17.5	14.5	6.1

Table 2: *Aedes aegypti* prevalence in two districts of Western Maharashtra

District	Locality (PHC)	*Aedes aegypti* prevalence		
		BI	PI	AHI
Satara	Nigdi	20.0	10.0	-
Kolhapur	Chandgad	35.0	35.0	10.0
	Hebbal	20.0	20.0	-
	Waghrali	30.0	20.0	20.0

BI = Breteau Index , **PI** = Premise Index , **AHI** = Adult House Index

Table 3: Survey of *Aedes aegypti* in Chikungunya affected areas in Kerala state

Sr. No.	Locality (PHC, District)	Premise Index (PI) %	*Aedes albopictus*	*Aedes aegypti*	*Aedes vittatus*
1	Vellapalam, (Attanikal, Malllapuram)	15.0 (3/20)	+	-	-
2	Kannakulum Tekkal, (Attanikal, Mallapuram)	20.0 (4/20)	+	-	-
3	Challipadan (Edavanna, Mallapuram)	25.0 (5/20)	+	-	-
4	Karakulam (Karulai, Mallapuram)	15.0 (3/20)	+	-	-
5	Edavanna (Edavanna, Mallapuram)	10.0 (2/20)	+	-	-
6	Iruvatti (Kavanoor, Mallapuram)	10.0 (2/20)	+	-	-
7	Kavanoor road (Atholi), Kozikodee	-	-	+	-
8	Atholi (Atholi, Kozikodee)	20.0 (4/20)	+	-	+
9	Kadalundi (Chaliyam, Kozikodee)	20.0 (4/20)	+	+	-

+ = Mosquito presence detected , - = Mosquito presence not detected

Table 4: Outbreak Investigation Studies conducted at Barsi (Maharashtra, October-2007): Details of the Sandfly collected

Locality	Species	Number
Khoslamb	Phlebotomus species (Male)	71
	Phlebotomus species (Female)	77
	Sergentomyia species (Male)	12
	Sergentomyia species (Female)	11
Surdi	Phlebotomus species (Male)	22
	Phlebotomus species (Female)	41
	Sergentomyia species (Female)	2
Khandre	Phlebotomus species (Male)	7
	Phlebotomus species (Female)	21
Kaligaon	Phlebotomus species (Male)	12
	Phlebotomus species (Female)	23
	Sergentomyia species (Female)	4
Total		**303**

Table 5 : Summary of Sandfly collections in the Nagpur division, Maharashtra:

Sr. No.	District	*Phlebotomus* species	*Sergentomyia* species	Total
1	Wardha	15	176	191
2	Nagpur	-	117	117
3	Bhandara	-	106	106
4	Gondia	6	95	101
5	Chandrapur	-	258	258
6	Gadchiroli	-	14	14
Total		21	766	787

Table 6 : Prevalence of Aedes aegypti in Pune Municipal Corporation

Sr. No.	Area	Breateau Index (BI)
1	Hadapsar	35.0 (7/20)
2	Kondhava	30.0 (6/20)
3	Ghorpadi	20.0 (4/20)
4	Guruwar Peth	- (0/20)
5	Phursungi	- (0/20)
6	Mundhava	15.0 (3/20)
7	Quarter gate	- (0/20)
8	Babajan Chowk	5.0 (1/20)
	Total	**13.1(21/160)**

Prevalence of *Aedes aegypti* in Pimpri-Chinchwad Municipal Corporation

Sr. no.	Area	Breateau Index (BI)
1	Kirkee	- (0/20)
2	Bhosari	10.0 (2/20)
3	Tathawade road	15.0 (3/20)
4	Ajmera Complex	- (0/20)
5	Kalewadi (Tapkir nagar)	15.0 (3/120)
6	Thergaon	5.0 (1/20)
7	Nigdi	- (0/20)
8	Kasarwadi	20.0 (4/20)
9	Chinchwad gaon	15.0 (3/20)
10	Dapodi	20.0 (4/20)
11	Nadhenagar	5.0 (1/20)
12	Yamuna Nagar	- (0/20)
	Total	**9.1 (22/240)**

Table *7* : Processing of Adult and Larval stages of *Aedes aegypti* for Dengue virus detection using IFA test

Sr. no.	Area	*Aedes aegypti* collected as Adult stage (processed individual head squashes)		*Aedes aegypti* collected as Larval/Pupal stage (processed by inoculation in *Aedes aegypti* mosquitoes)	
		Male	Female	Male	Female
1	Ghorpadi	0/5	0/7	0/93	0/57
2	Kondhava	-	0/9	0/25	0/40
3	Hadapsar	0/4	0/1	0/7	0/9
4	Mundhava	0/1	-	0/11	0/4
5	Dapodi	-	0/4	-	-
6	Kasarwadi	-	-	0/33	0/19

Service Provided

- **Detection of arboviruses from arthropod samples**

  Two Hundred and Three *Aedes aegypti* mosquitoes were received from local health authorities from Maharashtra (Ahmadnagar, Nasik, Latur) and Gujarat (Ahmadabad, Surat) states. Four mosquitoes from Ahmadabad were tested positive for Dengue virus.

Publications

- Mourya DT, Gokhale MD, Kumar R. Xenodiagnosis: Use of mosquitoes for the diagnosis of arboviral infections. **J Vector Borne Dis**, 2007 Dec; 44(4): 233-40.
- Kanojia PC, Jamgaonkar AV. Mosquito records from a hot and dry climatic area experiencing frequent outbreaks of Japanese Encephalitis, Bellary district, Karnataka, India. **J Am Mosq Control Assoc,** 2008 Mar; 24(1):6-10.

Participation in conference

- Studies on multiplication and transovarial transmission of a recent (2006) and an old (1964) strain of Chikungunya virus in *Aedes aegypti* mosquitoes, an abstract published by DT Mourya, A. Sarkar, MD Gokhale, S. Kumar, P. Barde, A. Sivram and R. Kumar in International conference of Emerging and Re-emerging Viral Diseases of Tropics and Sub-Tropics, December 11-14, 2007, New Delhi- 110012. Page-309.
- Prohibitin confers Dengue-2 virus refractivity in *Aedes aegypti*, an article published by Dilip N. Deobagkar, Anjali Apte, Mandar Paingankar and Mangesh Gokhale in Arthropod Borne Viral Infections: current status and research, in the eighth Sir Dorabjee Tata symposium, 2007.

Training given

Training was given to a M.Sc. Student from Bharati Vidyapeeth, Microbiology department for the completion of M.Sc. Project work.

Bioinformatics
&
Data Management

Bioinformatics & Data Management

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12 Bioinformatics & Data Management

- Evolutionary timescales of Chikungunya viruses inferred from the whole genome/ E1 gene
- Diversifying selection analysis of the Z genotype of Avian Influenza H5N1 viruses
- In-silico sequence and structure-based antigenic analysis of the G, N and M proteins of Chandipura virus

Evolutionary timescales of Chikungunya viruses inferred from the whole genome/ E1 gene

Sarah Cherian, AM Walimbe, AC Mishra and VA Arankalle

Introduction

The only report (Powers 2000), in the area of Chikungunya (CHIK) virus evolution is based on a phylogenetic analysis of partial E1 gene sequences and estimates an average divergence rate for the CHIK virus lineage. However, in this study, due to limited sequences available and analyzed one expects high degrees of errors as well as observes larger confidence intervals. With the current 2005-07 epidemic it becomes vital to reanalyze using larger data sets and accurate dating methods based on rigorous hypothesis testing and statistical approaches.

Analytical methods have been recently developed that explicitly account for lineage-specific rate through the use of a "relaxed molecular clock" (Drummond et al., 2006). Using these methods, we report here the mutation rates as well as infer the ancestral times based on the analysis of whole genomes as well as the analysis of the E1 protein sequences from the isolates belonging to the East Central South African (ECSA), West African and Asian genotype. An isolate of 2007 from the state of Kerala, which was severely affected, was sequenced as a part of this study and included in the current analysis. Seven additional whole genomes of CSF samples were also included in the present study.

Objectives

- To estimate the evolutionary rates and divergence times for Chikungunya genotypes
- To determine the time to the most recent common ancestor (tMRCA) of the isolates of 2006-08.

Work Done

Partial E1 gene sequences (n=67) of length 1044 nt and 27 whole genomes of CHIKV were analyzed in the present study. All sequences used had a known date of collection and 100% identical sequences were eliminated from the study. To test the molecular clock the following steps were followed. The Bayesian Markov Chain Monte Carlo (MCMC) approach available in the BEAST 1.4.5 package (Drummond and Rambaut 2007), was employed to estimate the rates of evolutionary change, the divergence times and the MRCA of the CHIK viruses. The relaxed clock (uncorrelated exponential) and the strict molecular clock models were implemented for both the partial E1 gene and the whole genome data sets. For each case, we applied the demographic models of constant population size and exponential population growth.

Between the constant population and the exponential growth population demographies, the constant population model was found to be more appropriate for the given data. Under the best-fit model (uncorrelated exponential, constant population size), the mean evolutionary rate of the 27 complete genomes was 7.8×10^{-4} subs/ site/year with the 95% CIs as $3\text{-}13.2 \times 10^{-4}$. The estimates for the time to the MRCA (tMRCA) of the different genotypes of CHIK for the uncorrelated exponential, constant population model for the whole genome dataset was almost 200 years with the upper bound as 350 years. The most recent common ancestor (MRCA) of the 2005-07 isolates had a mean age of about 9 years with 95 % HPD estimates of 3 to 16 years, interestingly from either of the datasets studied.

Summary

In the present study, the evolutionary timescales of CHIK viruses was estimated to be in the last 350 years with the progenitor of the 2005-07 epidemics existing around 9 years ago. The presence of a strain in India in 2000 that bears 99% identity with a Ugandan strain of 1982, which correlates with the tMRCA of the Indian and Indian Ocean isolates, confirms our earlier report that the progenitor of the 200507 isolates originates fromUganda's neighbourhood.

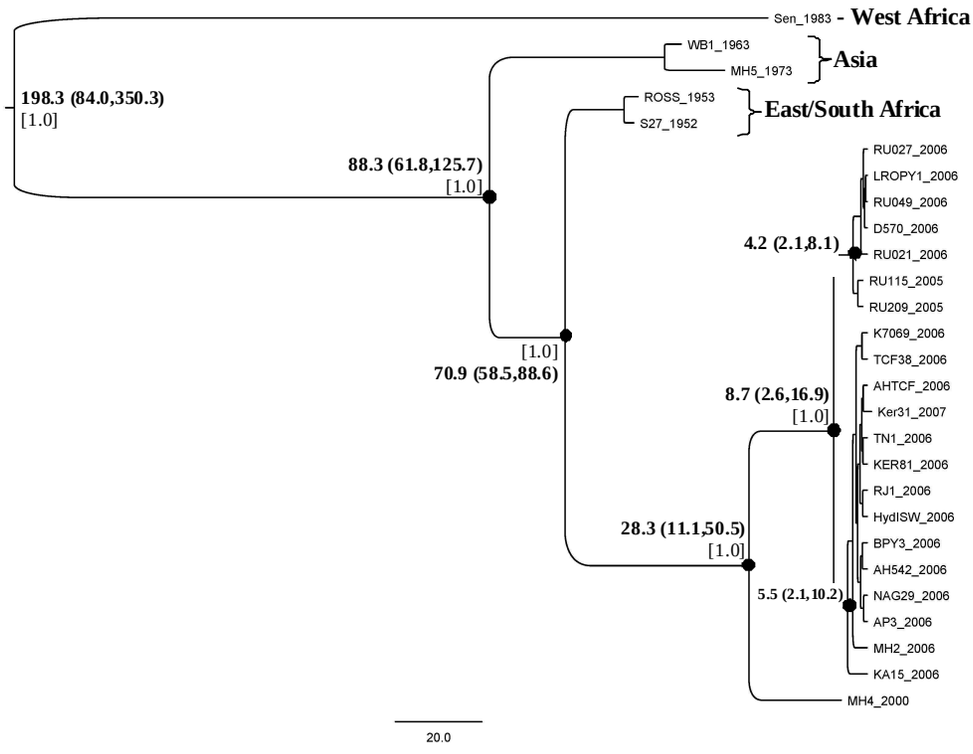

Fig. 1: Bayesian MCMC tree for Chikungunya whole genomes under the relaxed clock constant population model. Also shown are the posterior probabilities (in square brackets), estimated ages (bold letters)

Diversifying selection analysis of the Z genotype of Avian Influenza H5N1 viruses

Sarah Cherian, Santosh Jadhav and AC Mishra

Introduction

Since its emergence in 2002, the avian influenza A H5N1 genotype 'Z' has emerged as the dominant circulating strain in both aquatic and terrestrial poultry in eastern Asia. From 2003, the virus has also been responsible for outbreaks in over 30 countries in Central Asia, Middle East, Europe and Africa (WHO, OIE site). The genotype has evolved into two major clades, viz. Clade 1 and clade 2 with the latter distinguishing into six sub-clades (2.1 to 2.6). Genetic characterization of two full genomes of strains isolated from an outbreak of highly pathogenic H5N1 virus in poultry in India in 2006 was undertaken. Further, earlier studies (Li et al., 2004), based on the analysis of non-synonymous (d_N) and synonymous (d_S) substitution rates had suggested that the gene constellation of genotype Z viruses had not fully adapted to poultry and may continue to evolve through mutation or reassortment to achieve

greater viral fitness. Hence, the genes of the Avian Influenza H5N1 strains of the Z gentotype, circulating in 2005 and 2006 have been investigated for diversifying selection.

Objectives

- Phylogenetic analysis of all 8 gene segments for genotyping
- Diversifying selection analysis of Avian Influenza H5N1 isolates

Work done

Phylogenetic trees were developed for all the 8 gene segments using representative sequences of H5N1 currently circulating in Asia, Europe and Africa from the Genbank and Influenza Sequence Database. All the eight segments of the NIV isolates of the Manipur outbreak of 2007 and West Bengal outbreak of 2008 were Qinghai (QH)-like, clustering within Clade 2.2 of the Z genotype.

The homology modeling approach was used to predict the structure for the HA and NA sequences of the NIV isolate using the 2.95A^{o} X-ray crystal structure (2FKO) of hemagluttinin sharing 97% sequence identity and the 2.5A^{o} structure (2HU4) of neuraminidase of A/Vietnam/1203/2004 sharing 98% sequence identity respectively as templates. The Modeller8v2 was used for sequence alignment, model building and subsequent refinement of the model. For diversifying selection analysis, maximum likelihood models using CODEML in the PAML software package (Yang, 1997) were applied to sequences representing the clades 1 and 2 for all the gene segments. Tests for positive selection based on M7 and M8 codon substitution models were applied to 50 sequences representative of the clades 1 and 2. Though the mean values for all the genes were less than 1, suggestive of their overall conservative evolution, the tests for positive selection indicated that the HA1, M2, NA and NS1 genes showed significant evidence of positive selection (Table 1). Bayesian models identified several specific sites under positive selection with a high posterior probability in these gene segments. Many of the positively selected sites identified are associated with either mapped neutralizing epitopes, CTL epitopes or with regions involved in receptor-binding affinitiy. In general, amino acids at sites showing a very strong evidence of positive selection had higher physiochemical diversity compared with the sites showing weak evidence. Further, most of the positively selected sites were exposed. The fact that the positive selection sites could be mapped on to validated immunogenic epitopes indicates that the diversification enables evasion of the host immune response.

Table 1: Nucleotide substitution models, parameters of positive selection tests M7 & M8 & positively selected sites, for genes of H5N1 influenza viruses isolated in 2005-06 showing significant evidence of positive selection Bold font > 99% posterior probability. (GTR- General Time Reversible)

Gene region	Sequences (n), Length (bp)	Average (Li method)	Selected model, α parameter	Likelihood ratio and parameters	Positively selected sites
HA1	127, 969	ds = 0.133 dn = 0.021 ω = 0.158	GTR + γ α = 0.90	M7 lnl = -5967.3828 p = 0.020, q = 0.135 M8 lnl = -5937.832 p_0 = 0.989, p = 0.140 q = 1.334, p_1 = 0.010 ω = 3.05 p-value = <0.001	**86**(A,V,T,S), 123(S,A,P), **129**(S,L,A), **138**(Q,L,N,H,M), **140**(R,S,T,E,Q,N), **155**(N,D,S), **156**(A,T,S), 184(A,E,G)
NA	114, 1407	ds = 0.117 dn = 0.014 ω = 0.119	GTR +γ α = 0.44	M7 lnl = -7795.505 p = 0.059, q = 0.473 M8 lnl = -7787.097 p_0 = 0.996, p = 0.095 q = 0.855, p_1 = 0.004 ω = 2.77 p-value = 0.0002	44(R,H,C,Y,Q,N), 340(P,L,S,H,T)
M2	103, 291	ds = 0.032 dn = 0.018 ω = 0.56	K81+γ α = 0.223	M7 lnl = -1079.219 p = 0.005, q = 0.012 M8 lnl = -1064.537 p_c = 0.946, p = 26.896 q = 99.0 p_1 = 0.055 ω = 5.14 p-value = <0.0001	10(P,L), 18(R,K), **27**(V,A,I), 28(V,I,F), 31(S,N), 65(T,K,M), **66**(E,T,A,G), **82**(N,S), 95(E,K,Q)
NS1	108, 690	ds = 0.032 dn = 0.018 ω = 0.56	K81+γ α = 0.397	M7 lnl = -3484.53 p = 0.005, q = 0.016 M8 lnl = -3473.099 p_0 = 0.993, p = 0.011 q = 0.033, p_1 = 0.007 ω = 4.40 p-value = <0.0001	48(N,S), 86(A,T,V), **112**(T,A,E), **127**(T,N,D,V,A), **171**(N,G,E,D,S), 207(G,D,N), **209**(D,N,G), 212(L,P,S,F), 221(K,N,R,E)

Future plans

The subclades of the Z genotype would also be studied for diversifying selection analysis. The study would be useful in understanding the evolutionary patterns within the clades of the Z genotype.

In-silico sequence and structure-based antigenic analysis of the G, N and M proteins of Chandipura virus

Sarah Cherian and VA Arankalle

Introduction

The Chandipura virus (CHPV) is emerging as a fatal infectious disease with very high mortality rate. Hence it would be apt to use in-silico sequence and structure based approaches to determine epitope driven vaccine candidates. Among (CHPV) structural proteins, glycoprotein G, the spike protein protrudes externally from the outer membrane of the virus particle and elicits antibody response thus acting as a major antigenic determinant (Neumann et al. 2002). Matrix protein M lies in the inner surface of the virion to tether the nucleocapsid to the membrane. L and P are packaged within the mature virion and remain associated with the core nucleocapsid particle. The. Nucleocapsed (N) binds with nascent leader RNA and initiates encapsidation.

Whole genome sequence data is available for CHPV isolates from febrile as well as encephalitis cases. As far as 3D structures are concerned, the homologous crystal structures available are that of the pre and post fusion forms of the Vesicular Stomatitis Virus glycoprotein G, matrix protein of the Vesicular Stomatitis Virus (VSV) and a recent crystal structure of the Rabies virus nucleocapsid 'N' protein complexed with RNA. The G and N proteins are known to have immunogenic characteristics. Hence the sequential B - cell epitopes as well as the T - cell epitopes on these proteins can be predicted. Peptides containing these antigenic regions may help in the design of subunit vaccines.

Objectives

- Modeling of the G, N and M proteins
- Prediction of T-cell and B-cell (linear and conformational) epitopes

Results

Sequence alignment of the G protein sequences of viruses of the rhabdoviridae family including the sequences of the CHPV isolates available has been carried out ensuring the alignment of conserved cysteine residues. Mapping of the known antigenic sites of VSV and Rabies virus indicates that certain mutations of CHPV isolates fall in the known antigenic sites. The tertiary structure models of the CHPV G and N **(Fig. 2)** proteins, by homology modeling approaches with Modeler in Discovery studio, using the VSV 'G' and 'N' proteins respectively as a template is made. Scan Prosite is used to locate functional motifs in the CHPV sequences of the G, M, and N proteins for characterization of the genome.

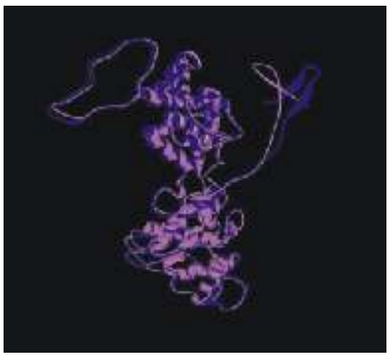

Fig. 2. Homology model of the CHPV 'N' protein (pink ribbon) superimposed on the template 'N' protein (blue ribbon) of VSV

Future plans

Characterization of the complete genome would be completed. Further, we would use immunoinformatics approaches to identify highly conserved Class II (Th) Chandipura epitopes in the G and N proteins. This could find applications in the development of a multi-epitope Chandipura "peptide" vaccine that may be used alone or in conjunction with the candidate preventive killed virus vaccine. Also, structure based drug design approach would be explored to determine candidate antiviral inhibitors.

Services Provided

Statistical data analysis

Regular services to the various experimental groups at NIV and MCC, Pashan, in the areas of statistical data analysis of Epidemiological and serological data, bioinformatics sequence analysis, phylogeny etc.

Computer related services

Management of the LAN and Internet services at NIV, Pune and MCC, Pashan - Mr. Vijayasimha.

Publications

- Arankalle VA, Shrivastava S, Cherian S, Gunjikar RS, Walimbe AM, Jadhav SM, et al. Genetic divergence of Chikungunya viruses in India (1963-2006) with special reference to the 2005-2006 explosive epidemic. **J Gen Virol**. 2007 Jul; 88(Pt 7): 1967-76.
- 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

Sarah Cherian

- Poster entitled "Patterns of diversifying selection in the avian influenza A H5N1 strains belonging to the genotype 'Z'" , Sarah Cherian, Santosh Jadhav, K. Anbarasu et al., 'Options for the control of Influenza V1', Toronto, Canada, June 16-23, 2007.
- MBL Workshop on Molecular evolution, Woods Hole, Boston, Massachusetts, USA, 23 July August 3, 2007.
- Invited talk: Selection Pressure Analyses of the Genes of AI A (H5N1) Genotype Z Viruses, International Symposium on Avian Influenza: Epidemiologic, Basic and Applied Research, New Delhi. India, October 29-31, 2007

- Attended Int. Conf. on Emerging & re-emerging viral diseases in the tropics & sub-tropics, New Delhi, 11-14 Jan. 2008.
- Invited talk: "Evolutionary dynamics of Chikungunya viruses", IInd CMC-NIV Symposium on Respiratory, Gastrointestinal, Arthropod and blood borne viruses, March 5, 2008

Atul Walimbe

- Poster presentation entitled "Molecular Evolution study of the Chikungunya virus isolates: (1952-2006)" at "13th International Bioinformatics Workshop on Virus Evolution and Molecular Epidemiology", held at Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Lisbon, Portugal organized by Rega Institute & University Hospitals, Leuven, Belgium, 9-14 September 2007.
- Capacity Building Workshop on "Application of Random/Mixed Effects Models" organized by ICMR, Dept. of Biostatistics, SGPGIMS in collaboration with NACO, WHO and CDC. held at Biostatistics Department, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, 10-12 December 2007.

K. Vijayasimha

- Advanced training in Statistical Package for Social Sciences, Bangalore, Sept. 3-7, 2007.

Santosh Jadhav

- ICMR Training program in Bioinformatics, Institute of Bioinformatics and Applied Biotechnology, Bangalore, Aug. 3-25, 2007.

Arpita Banerjee

- Joint workshop on "Knowledge discovery in Life sciences: Modeling small molecules to macromolecules" Bioinformatics centre, University of Pune, December 11-17, 2007.

High Containment Lab
and
Virus Repository

High Containment Lab and Virus Repository

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Mr. Sandeep Kumar	Ph.D. Student
Mr. R Mehla	Ph.D. Student
Ms. A. Sivaram	Ph.D. Student

13 High Containment Lab and Virus Repository

- Establishment of PCR based diagnostic tests for emerging viruses
- Multiplication of 17D virus in Indian strain of *Aedes aegypti* mosquito
- Development of serological and molecular diagnostic facility for Kyasanur Forest Disease virus
- Molecular characterization of viruses (Family: Bunyaviridae) isolated from India
- Molecular characterization of dengue virus isolates from different parts of India
- Studies on the interaction of densovirus (DNV) with the susceptibility of *Aedes aegypti* mosquitoes to Chikungunya virus
- Establishment and characterization of a new *Aedes aegypti* cell line
- Growth kinetics of Chikungunya virus in different cell lines
- Development of an inactivated Chikungunya virus vaccine

Establishment of PCR based diagnostic tests for emerging viruses

DT Mourya, Sandeep Kumar, PD Yadav and PV Barde

Introduction

With a view biodefense preparedness, last year RT-PCR tests were established for certain known threats of viruses like Crimean Congo Hemorrhagic Fever, Rift valley Fever and Lassa virus. Hantaviruses are transmitted to humans who inhale aerosolized excreta from chronically infected rodents. Infection with certain Hantavirus species can manifest in humans as Hantavirus pulmonary syndrome (HPS) or hemorrhagic fever with renal syndrome (HFRS). Three main groups of Hantaviruses exist, corresponding to viruses vectored by Murinae, Arvicolinae, and Sigmodontinae subfamily rodents. This year attempts were made to establish molecular diagnosis, which can detect all three groups of Hantaviruses.

Objective

- Establishment of PCR based diagnostic test for certain viruses for bio-preparedness

Work done

In this connection, Nested RT-PCR tests were established for Murinae specific Hanta viruses based on the Nucleocapsid gene and RNA polymerase gene. Positive control RNAs were extracted form the gamma irradiated material received from CDC, Atlanta. RNA polymerase (600 bp) and Nuclecapsid gene (1080 bp) were amplified by a single step RT-PCR. Purified PCR products were cloned using TOPO-XL kit (Invitrogen). Two positive clones were grown in bulk in broth and plasmids were purified. Similarly, amplicons obtained from PCR for glycoprotein gene (M) of Ingwavuma virus, which is highly conserved among Bunyaviruses, was used for cloning in for positive control. Positive control RNAs were extracted from the isolates received from NIV repository.

Multiplication of 17D virus in Indian strain of *Aedes aegypti* mosquito

DT Mourya

Introduction

It has been long suspected that the Indian *Aedes aegypti* strains are probably not susceptible to yellow fever virus, with this view Pilot experiments were conducted with vaccine stain to understand this phenomenon.

Objectives

- Generating baseline information on certain viruses for bio-preparedness

Work done

Experiments were carried out to determine if Indian strain of *Aedes aegypti* mosquitoes support the multiplication of 17D vaccine strain of this virus. Experiments were conducted in BSL-3 facilities where *Aedes aegypti* mosquitoes were infected by intrathoracic inoculation. The dengue-2 infected mosquitoes were used as control in the test. The immunofluorescence assays carried out using dengue hyper immune serum (with the hypothesis that the flavivirus cross reactivity of the immune serum will help in identifying/detecting virus multiplication). The head squashes of mosquitoes prepared after 8 post infection days showed that the vaccine strain of virus multiplied in these mosquitoes.

Development of serological and molecular diagnostic facility for Kyasanur Forest Disease virus

DT Mourya, PD Yadav, PV Barde, R Mehla and JP Thakare

Introduction

Shimoga district, Karnataka State in India is the only geographical area in this country, which is endemic for this disease. In India, other flaviviruses are also known to cause haemorrhages.

Early symptoms of KFD mimic those of other hemorrhagic diseases such as Japanese encephalitis, dengue and enteric diseases prevalent in India. In the past, HI, CF and in vivo inoculation of clinical samples into mice were the available tests for diagnosis. These tests are time consuming and their sensitivity depends on the presence of higher quantity of virus in inoculum for mice or higher quantity of antibodies in the patient's serum. In the case of a KFD infection, the viremia in KFD patients lasts for 12-13 days of illness and during the first 3 to 6 days the viremia remains considerably high with titers as high as 3.1×10^6, unlike most other arboviruses. Therefore this year efforts were made to establish serological assays as well as diagnostic Taqman based real time PCR for diagnosis, which discriminated other flaviviruses prevalent in this country.

Objectives

- Establishment of diagnostic methods for Kyasanur Forest Disease virus.

Shimoga district, Karnataka State in India is the only geographical area in this country, which is endemic for this disease. In India, other flaviviruses are also known to cause hemorrhages. Early symptoms of KFD mimic those of other hemorrhagic diseases such as Japanese encephalitis (JE), dengue (DEN) and enteric diseases prevalent in India. The differential diagnosis of this disease has therefore, become a challenge. In the past, haemagglutination inhibition or complement fixation test and *in vivo* inoculation of patient's sera into suckling mice were the tests of choice for diagnosis.

Work done

A) Validation of Taqman based Real time PCR for the diagnosis of KFD

As reported earlier, we had developed an RT-nPCR and Taqman based real time RT-PCR assay for the diagnosis of Kyasanur Forest Disease virus (KFDV) based on conserved NS5 gene. It was found specific and sensitive, and could detect up to 38 RNA copies of KFDV RNAs. Both the assay could detect KFDV RNA from human clinical samples and can provide an early and accurate diagnosis for this infectious disease agent of biosafety level-3. Subsequently, diagnostic RT-nPCR and Taqman real time PCR was performed on the recent 10 KFD isolates and 11 acute stored human serums (2 to 7 post onset day) samples suspected for KFDV. Out of these, 7 serum samples and all the 10 isolates were found positive by both the assay (Table 1). Four serum samples were found negative. In the first PCR, we could not detect any product with serum sample except one sample (A001), which gave product in the first PCR of proper size, however, using the nested PCR viral RNA could be detected in seven serum samples. When same RNAs were subjected to single step Taqman RT-PCR, it was less sensitive at least by one log. It clearly indicates that two-step will be a better method for clinical sample because of low availability of sample and the same cDNA of real time can be use for nested PCR and sequencing using same diagnostic set of primers.

Table 1: Comparative analysis of suspected KFDV acute-phase serum samples for positivity by nested RT-PCR, virus isolation, and real time RT-PCR detection

Sample No.	Diagnosis of clinical samples			Diagnosis of infected Mouse brain suspension	
	1st RT-PCR	Nested PCR	Real time PCR	1st RT PCR and Nested PCR	Real time PCR
A001	+ve	+ve	+ve	+ve	+ve
A002	-ve	+ve	+ve	+ve	+ve
A005	ND	ND	ND	+ve	+ve
A006	-ve	+ve	+ve	+ve	+ve
A007	ND	ND	ND	+ve	+ve
A010	-ve	+ve	+ve	+ve	+ve
A018	-ve	+ve	+ve	+ve	+ve
A020	-ve	+ve	+ve	+ve	+ve
A021	ND	ND	ND	+ve	+ve
A023	ND	ND	ND	+ve	+ve
A008	-ve	-ve	-ve	-ve	-ve
A019	-ve	+ve	+ve	Isolation ND	Isolation ND
A012	-ve	-ve	-ve	-ve	-ve
A013	-ve	-ve	-ve	-ve	-ve
A029	-ve	-ve	-ve	-ve	-ve

ND = Not done as it was not available

B) Development of MAC ELISA

In continuation of the work done in developing the MAC ELISA test for detection of KFDV, the reagents prepared earlier were tested for their activity using direct and indirect ELISA tests. Attempts were also made to see the feasibility of establishing ELISA test using flavivirus cross-reactive monoclonal antibodies [clones: I D 4; II B1B7; II B1E7 and V F6 G5]. All the four-ascietic fluids did not react to KFDV proteins from mouse brain and infected tissue culture fluid even at a 1:2 dilution. Effort was also made to establishment of standardized serological procedures, like Haemagglutination (HA) and mouse brain derived antigen preparation by sucrose-acetone extraction methods for KFDV. Immune serum was prepared in rabbit using semi-purified KFDV showed reactivity up to 1:800 dilutions. The IgG from this was purified and was tagged to HRP for future use as probe. Further development of MAC ELISA is in progress.

Molecular characterization of viruses (Family: Bunyaviridae) isolated from India

PD Yadav and DT Mourya

Introduction

In India, emerging viral infection is a reality. The emerging and re-emerging viral threat is posing a major public health challenge. In India, during the past 5 decades, several viral epidemic investigations have been carried out and several viral isolates were obtained. Of these viral isolates some were identified as etiological agents, which were mainly responsible for causing human disease, however, during such investigations several viruses were isolated which were either from humans or from domestic animals and blood sucking arthropods, which were present in the surrounding ecosystem. Proper identification and establishing quicker diagnostic methods help in understanding their exact role in the diseases burden caused by these viruses and their possible role as emerging as etiological agents in the form of epidemics. The present project focuses on the identification of viruses isolated during the past and currently placed in the family Bunyaviridae, based on serological methods.

Objectives

- Characterization of unidentified Bunyaviruses at molecular level to assist in right taxonomic placement.

Characterization of Chittoor virus based on nucleocapsid and glycoprotein gene

In India, Chittoor virus was isolated from mosquitoes and identified and classified as Batai virus serogroup based on serological study. In nature this virus is isolated from *Anopheles* and *Culex* mosquito in India but Chittoor virus also multiplies in *Ae aegypti* mosquitoes after intra thoracic inoculations according to our laboratory study.

A) Complete genome sequencing of Chittoor virus

Work Done

All the three strains (804986, 804988 and 804992) of this virus were propagated in Vero-E6 cell line. CPE was observed and cells were harvested at 4th PID. In this study, we have amplified complete Glycoprotein, complete Nucleocapsid gene and partial L gene to characterize three strains of Chittoor virus. Nucleocapsid (N) gene, which encode for nucleocapsid and nonstructural genes and M gene encodes for glycoprotein gene were amplified using conserved Bunya genus specific N (970 bp) and M (4400 bp) specific primers by a single step RT-PCR. PCR products were purified and sequencing has been performed with different sets of primers of N and M gene. In both the cases internal primers are being designed to get complete sequence.

All the sequences of Batai virus were analyzed using Clustal*W* and phylogenetic tree were made using neighour joining algorithm with 1000-boot strap. The phylogenetic result of N gene showed that all the Batai viruses make one monophyletic tree and a very high level of conservation is recorded in all the three segments. A very high level of similarity is recorded in all four isolates of Chittoor virus, from India. Ngeri virus and Garisa virus which are a close relative of Chittoor virus and there is evidence found of reassortment where their N and L gene resemble to Batai virus while M gene is similar to some unknown Bunyavirus. In the case of Indian isolates, as it is reported for Garissa virus M gene is encoding for 165Kda of protein of 1464 aa long ORF. N gene is encoding for 319 aa proteins of 35.8kDa.

The phylogenetic analysis of N gene showed that all Batai viruses are highly conserved at nucleotide level and there is not a single change at amino acid level, it seems that one single virus strain is circulating in India and it is evolved with nature in such a way that in spite of isolating from different mosquitoes species there is not much change at evolutionary level with time in viral RNA. Similar results were found with the M genome of Batai virus, which also showed high conservation at nucleotide and amino acid level. Further analysis is in progress.

B) Thimiri virus (66414) confirmation as Simbu group of virus

This virus was isolated from *Ardeola grayi*, paddy bird in 1963, Tamil Nadu, India and based on the serological data (CF and HA), this virus was confirmed as a member of Simbu serogroups.

Work Done

Propagation and confirmation of Thimiri virus as Simbu group of virus

Virus was propagated in *Vero E-6* cells by infecting with reconstituted virus suspension after filtration, which showed CPE at 46 hour. Cytopathic pattern of the virus was rounding and shining of the cells and detachment of cell sheet. Conserved sets of primers tried for amplification of partial glycoprotein G2 gene. Sequencing data confirmed that this virus belongs to Simbu group of virus and showed maximum homology with Simbu group of viruses in which there is highest identity with Jatobal virus. Based on the serological relationships, viruses in the genus Bunyavirus have been divided into 18 serogroups. One of the largest serogroups within the genus is the Simbu serogroups, which is further divided into five serocomplex, Simbu, Manzanilla, Oropouche, Thimiri and Nola.

C) Identification of Umbre virus as Bunya viruses at molecular level

Three isolates of this virus were from mosquitoes, collected from Vellore taluka of north Arcot district. During 1955-1957, five more strains (G7441, G83335, G9601, G7441 and G 16310) were isolated from *Culex vishnui* complex pool.

Work Done

Seven isolates of Umbre virus, which were serologically identified and placed in Turrlock serogroup in the past, procured from NIV registry, were propagated in *Vero E-6* cell line. CPEs was observed at 4th PID. Cells were harvested further for identification of this virus, RNA was isolated and a partial conserved M gene of Bunyamwera genus specific RT-PCR performed and after getting desired size of products, purification of products and sequencing was done. Different sets of primer tried and successfully partial M gene of 575 could amplify using partial M gene conserved primers with a single step RT-PCR with modified RT-PCR conditions. No Turlock serogroups sequence is known in Gene bank and this will be the first report of identification of this virus at molecular level.

Phylogenetic analysis of 575 bp of glycoprotein gene (G2) data showed that the entire Umbre virus is making a monophyletic tree where all the seven isolates fall in the one phylogenetic lineage. Only one of the isolates 809365 is showing some difference as compared to other Umbre virus. This virus is showing maximum homology with Kaeng Khei virus that represents a distinct group within genus Orthobunyavirus **(Fig 1)**. Further analysis is in progress.

D) Confirmation of Balagodu virus (633970) as Shimbu group of virus based on partial glycoprotein and complete Nucleocapsid gene

This virus was isolated from KFD-endemic area, Sagar Taluka, Shimoga district, Mysore State from a paddy bird, *Ardeola grayii* (Skykes) in 1969. Biological transmission of this virus was recorded in *Culex quinquefaciatus* mosquitoes but not with ticks (*Haemophysalis spinigera*).

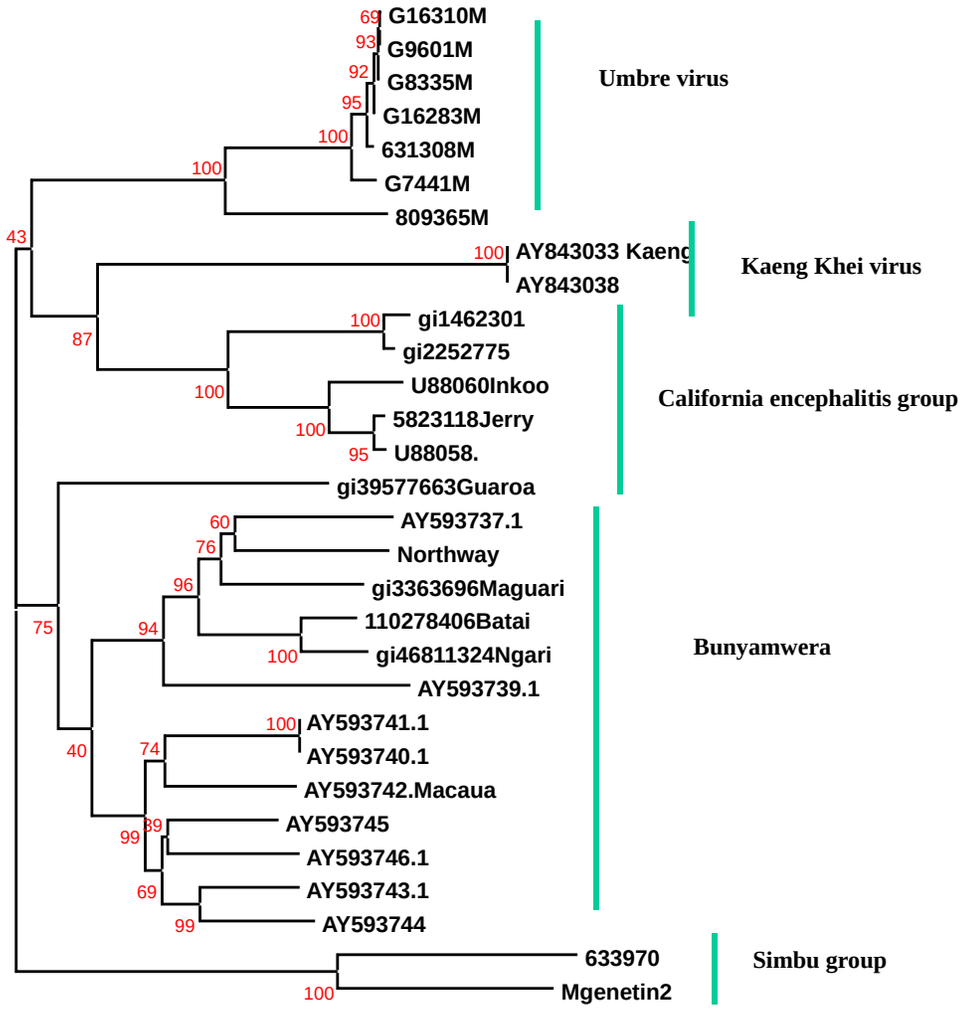


Fig. 1: Phylogenetic tree showing similarity between Umbre and other Orthobunyavirus

Work done

Virus was revived in infant *Swiss albino* mice and infected by intra cerebral route. Sick mouse brains were harvested and 10% mouse brain suspension prepared in BAPS was directly used for RNA extraction using Tripure (Roche) and RNaid kit (Biogene) according to the manufacturer's instructions. A 970 bp of complete Nucleocapsid (N) gene was amplified using genus specific conserved primer, which includes nucleocapsid and nonstructural gene in overlapping frame. Glycorprotein gene G2 partial fragment of 575 bp could also amplify by a single step RT-PCR at similar RT-PCR condition (50^{0}C-30 min for RT, and PCR was for 94^{0}C - 2 min, 94^{0}C -15 sec, 50^{0}C 30 sec and extension of 2 min at 68^{0}C for 39 cycles.

Complete N and partial M gene was sequenced and sequences were assembled and curetted using software KODON. N gene of virus was sequenced which showed maximum homology with Ingwavuma virus while M gene sequence showed maximum homology with Oropouche virus. In Gene bank, only two N gene sequence of Ingwavuma virus are available but no sequence is available for glycoprotein gene of this virus.

E) Complete Nucleocapsid gene sequencing of Ingwavuma virus

Earlier we have reported the identification of two Ingwavuma virus (strain No. 86209 and 86627) based on partial nucleocapsid gene (750 bp) and partial M gene. Further primer designed and complete N gene of 975 bp is sequenced which encodes for nucleocapsid gene and nonstructural gene in overlapping frame. To complete the whole sequence, N gene was amplified with the help of genus specific primer and cloned into TOPO-TA vector and sequenced with the help of vector primer and N gene primers. Nucleocapsid open reading frame start at position 42 and end at 743 nucleotide, which encodes for 248 amino acid. N genome is encoding for 35.8-kDa proteins. Nonstructural gene is 232 nucleotide long.

F) Development of Nested RT-PCR to detect Ingwavuma virus

Based on the sequences of Nucleocapsid gene available in data bank, a diagnostic nested RT-PCR is developed to detect ING virus. First RT-PCR primer is designed based on conserved primer of Bunyavirus genus. This can detect any Simbu group of virus from Bunyavirus group. Then further two forward and two reverse primers were designed to detect only ING virus based on specific ING virus sequences. These primers were used in four different combinations to see the sensitivity of the test. One of the primers set was found most sensitive for nested PCR.

G) Screening of Pig sera samples for Ingwavuma viral RNA

In India, this virus has also been isolated from a pig from Karnataka state. To determine whether this virus is circulating in pigs, 25-serum sample from Karnataka were screened for ING virus by RT-PCR. One of the samples was found positive, the sequencing data showed 95% similarity with Simbu group of virus; further work is in progress to confirm its identity **(Fig 2)**.

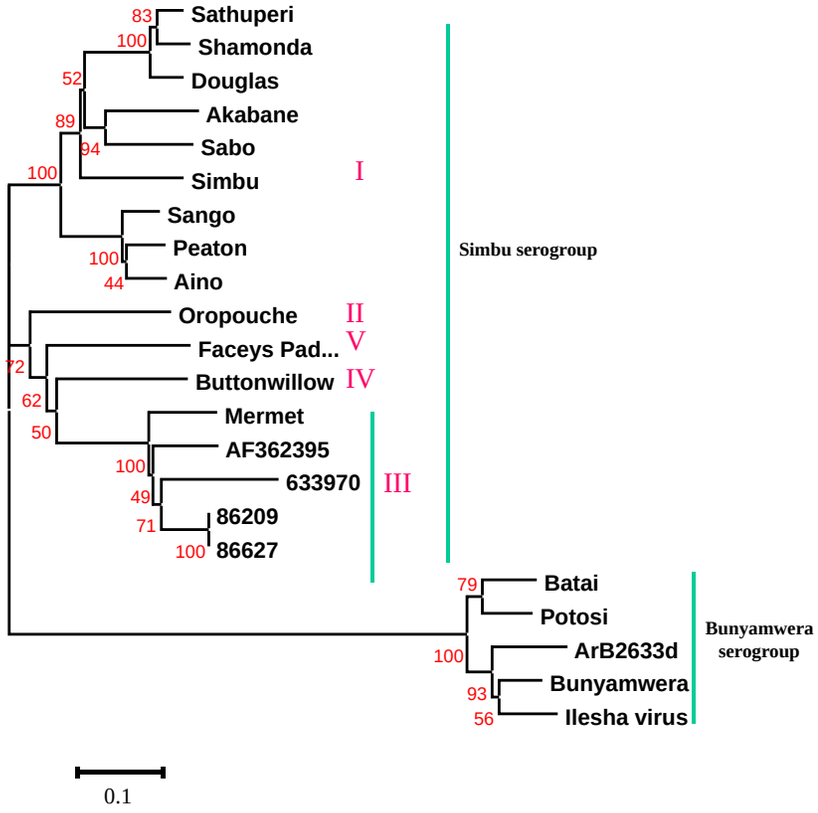


Fig. 2: Phylogenetic tree of Ingwavuma virus by NJ method at 1000 BT with other Orthobunyaviruses

Molecular characterization of dengue virus isolates from different parts of India

Sandeep Kumar and DT Mourya

Introduction

In the past few decades the epidemiological picture of DEN has changed in India. The incidence, distribution particularly in rural areas and clinical severity of DEN has increased dramatically. Population growth provides many susceptible hosts. Uncontrolled urbanization leads to inadequate management of water and waste, providing a range of large water stores and disposable, non-biodegradable containers that become habitats for the larvae. Besides this better mode of conveyances and tar-roads has increased movement of people, which has resulted in a proliferation of vector in newer areas and increased DEN cases. Therefore, the present study was undertaken to understand the phylogeny of DEN virus strains from India, isolated during the last few decades.

Objectives

- Genotyping of dengue isolates (E & NS1 gene) to understand variation in the strains

Work done

A) Genotyping of dengue isolates

Dengue fever (DF), a mosquito-borne viral infection caused by dengue (DEN) virus, is emerging rapidly in the tropical and subtropical countries of the world. This study reports the phylogenetic analysis, selection pressure and time scale evolution of twenty four DEN 2 virus strains, isolated during different epidemics of DEN viruses in India over a time span of almost 50 years. For this study complete envelope (ENV) and non-structural 1 (NS1) gene were studied. All of these strains were isolated from human samples two strains (715541 and 051774-1), which were isolated from *Aedes aegypti* mosquitoes in the 1971 and 2005 respectively. The lyophilized viruses were reconstituted and revived by inoculating in Swiss albino suckling mice by intra-cranial (IC) route. Ten percent mice brain suspensions were made in 0.75% BAPS and were stored in 70^0 C until used. RT PCRs were done on these MBR suspensions and sequences were analyzed after manual curetting.

The selection pressure analysis revealed the presence of positive selection at various sites in the ENV gene, whereas no site under positive selection with significant probability has been observed in NS1 gene. The rates of nucleotide substitutions were consistent among both the genes. The estimate of most recent common ancestor for DEN 2 viruses in India dates back to the beginning of 20^{th} century **(Fig 3 & 4).**

Fig. 3: The Phylogenetic tree of DEN 2 viruses on the basis of complete ENV gene sequences.

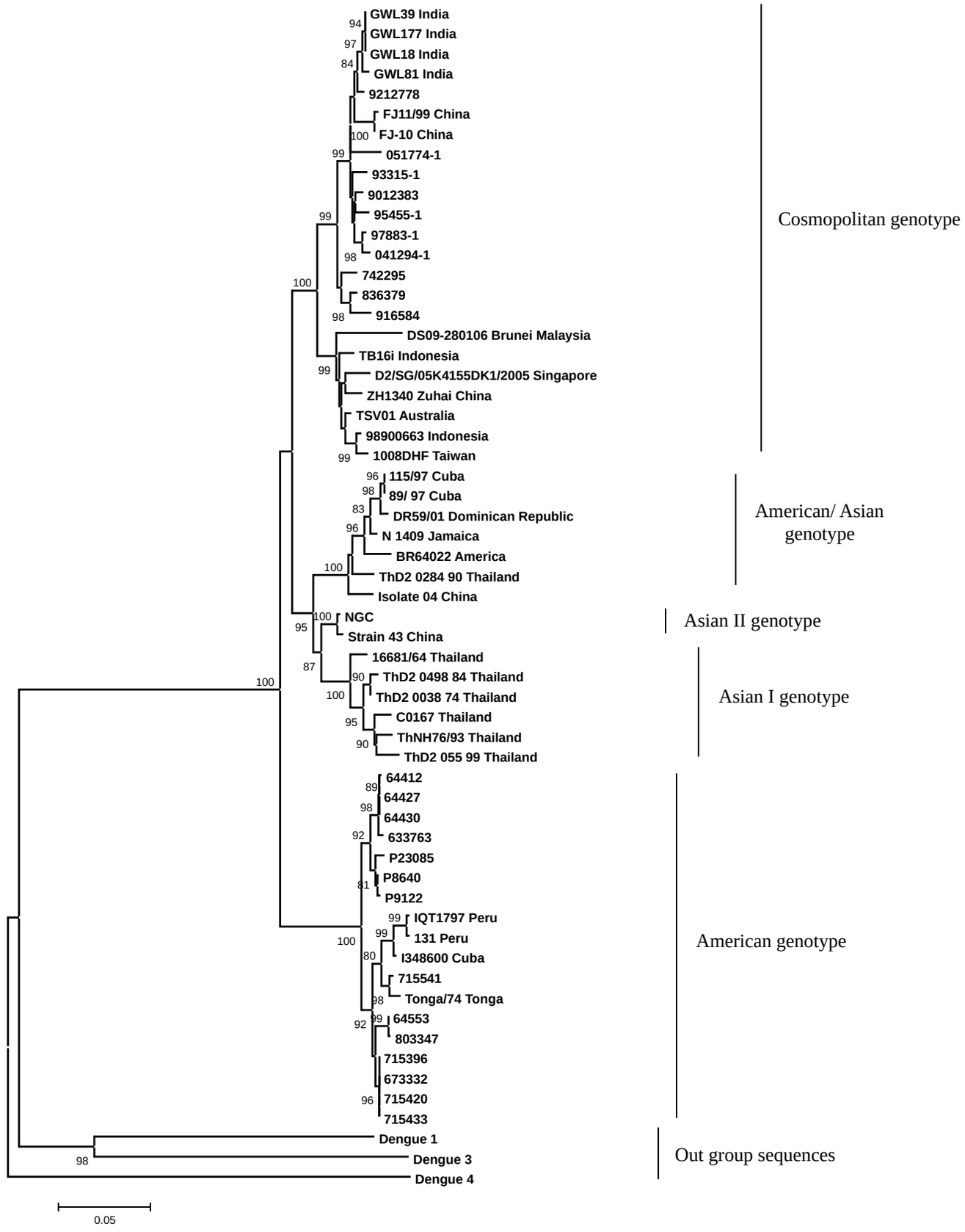


Fig. 4 : The Phylogenetic tree of DEN 2 viruses on the basis of complete NS1 gene sequences.

B) Development of SYBR green I based Real time RT PCR for detection of all four serotypes of dengue virus from mosquitoes

Rapid detection and accurate diagnosis of DEN along with serotype from the mosquitoes can help in both patient care as well as prevention and spread of the disease to newer areas. Infecting mosquitoes by intra-thoracic inoculation or infection of C6/36 *Aedes albopictus* cells and antibody staining are routine laboratory tests for establishing identity of dengue.

At present, indirect immunofluorescence (IFA) of the head squash of the mosquito using polyclonal immune serum is the method of choice at our institute. This method although is sensitive and reliable, does not determine serotype of the virus, and confirmation of virus serotype/s requires virus isolation and then further studies either molecular or serological, are time consuming. Here we report a SYBR Green I based real-time PCR method that we found is sensitive, accurate and fast for detection of DEN and its serotype from mosquito based on the melting temperature of obtained products.

The primers were designed from 3'NTR region of the DEN, which would pick-up all four serotypes. The virus stocks of all four serotypes were diluted in such a way that they have 10,000 infective particles per ml. These were used to infect the mosquitoes through intra-thoracic inoculation. The mosquitoes were stored on 2^{nd}, 4^{th}, 6^{th} 8^{th} and 10^{th} post infection day in liquid nitrogen till they were tested either by IFA or real time PCR tests.

Results of the studies are compiled in table-2, which clearly demonstrated that real time RT PCR is more sensitive, rapid and accurate test for detection of DEN from mosquitoes.

Table 2 : Percent positivity of DEN viruses (1 to 4) detected in inoculated mosquito with SYBR green I Real time assay at different PID

PID*	DEN 1 (%)	DEN 2 (%)	DEN 3(%)	DEN 4 (%)
2	33.3	50	16.6	33.3
4	66.6	83.3	33.3	66.6
6	66.6	100	66.6	66.6
8	83.3	100	83.3	100
10	100	100	100	100

Project concluded.

Studies on the interaction of densovirus (DNV) with the susceptibility of *Aedes aegypti* mosquitoes to Chikungunya virus

DT Mourya, MD Gokhale, PV Barde, A. Sivram and PD Yadav

Introduction

Recent reports have shown that presence of DNVs in the vector mosquitoes reduces the multiplication of dengue virus. DNVs are the etiologic agents of insect diseases known as densonucleosis, which is characterized by densely staining nuclei of infected cells and leads to either death or loss of vital functions in all stages of infected organisms. The first mosquito densoviruses discovered in a laboratory colony of *Ae. aegypti*, was named *Ae. aegypti* densovirus. Many aspects of virus pathogenesis and their interaction with alphaviruses and flaviviruses with regard to the susceptibility of mosquitoes need to be investigated. Present study was carried out to understand whether presence of DNV can also reduce the multiplication of Chikungunya virus.

Objective

To determine that multiplication of CHIK virus be affected by replication of MDV in the vector mosquito.

Work done

Mosquito densoviruses belongs to family Parvoviridae, sub-family Densovirinae genus Brevidensovirus. They are known to cause mortality in mosquitoes. The investigations on various *Aedes aegypti* mosquito populations collected form India showed presence of denso virus in some of the populations. The densovirus was isolated and the identity of the isolate was confirmed by PCR using densovirus specific primers and electron microscopy. Laboratory experiments showed high mortality in the batches of *Ae. aegypti* treated with higher concentrations of virus. The mortality was very high in unnatural hosts like *Culex tritaeniorhynchus*. The 1097 bp genome sequence showed 97% identity with that of densovirus reported from *Ae. aegypti* densonucleosis virus.

A) Detection of DNV in mosquito from different geographical locations in India

The presence of MDV in different mosquito populations was determined by PCR using the densovirus specific primers. Of the 30 different populations tested, 13 populations were found positive for this DNV.

B) Screening of mosquitoes for MDV from the Chikungunya affected areas

Recently, NIV had investigated Chikungunya (CHIK) virus outbreaks in Kadappa, Kurnool, Anantpur, Nalgonda and Chitoor districts. During investigation, mosquito pools were brought from these areas for the detection of CHIK virus. These pools were also screened for the presence of DNV. Out of 14 pools, 12 were positive for CHIK virus however out of these 12 pools, five were also found positive for DNV. These were from Kadappa and Chittor districts. The CHIK negative pools were also found negative for DNV.

C) Complete genome sequencing of DNV

Complete genome sequencing of DNV is being attempted from mosquito *Aedes aegypti.* We have got complete genome without cloning using primer walking in the genome. DNV virus of 3776 bp is sequenced except 3' UTR region that could not sequenced because of palindrome sequences. 5 UTR was also a challenge but using different denaturing condition it could be sequenced by using different sets of primers.

The composition of genome is A-21.2%, T-41.3%, G-19.5%, C-18.0% which encodes three open reading frame in overlapping fashion called as Left ORF, Mid ORF and right ORF of 849 amino acid, 358 aa and 362 aa respectively. 5 UTR is 164 nucleotide long. The molecular weight of Left, mid and right ORF is 97.5, 40.5 and 26.3 kDa in which first ATG starts at 129, 2561 and 2443 nucleotide position respectively. Left ORF very likely encodes for the nonstructural NS-1 protein since it contains the highly conserved NTP-binding amino acids domain of all parvoviruses. This will be the first genome of Denso virus from India isolated from *Aedes aegypti* mosquitoes. 8 AATAAA sequences corresponding to potential cleavage and poly adenylation sites for messenger RNA are present in the genome of DNV virus. The genome organization is very similar to the earlier published described genome of *Ae. aegypti* DNV. DNV virus aligned with other densoviruses available in Gene bank using ClustalW and Mega 4.0 software at 1000 bootstrap replicates using Neghour joining algorithm. The phylogenetic data showed that this virus is making a separate phylogetic lineage with *Ae. aegypti* DNV and *Ae. albopictus* DNV genome (**Fig.5)**. The DNV genome is showing 93% and 80% homology with *Aedes densonucleosis* virus (M37899.1) and *Ae. albopictus* DNV genome. Further analysis of this data is in progress.

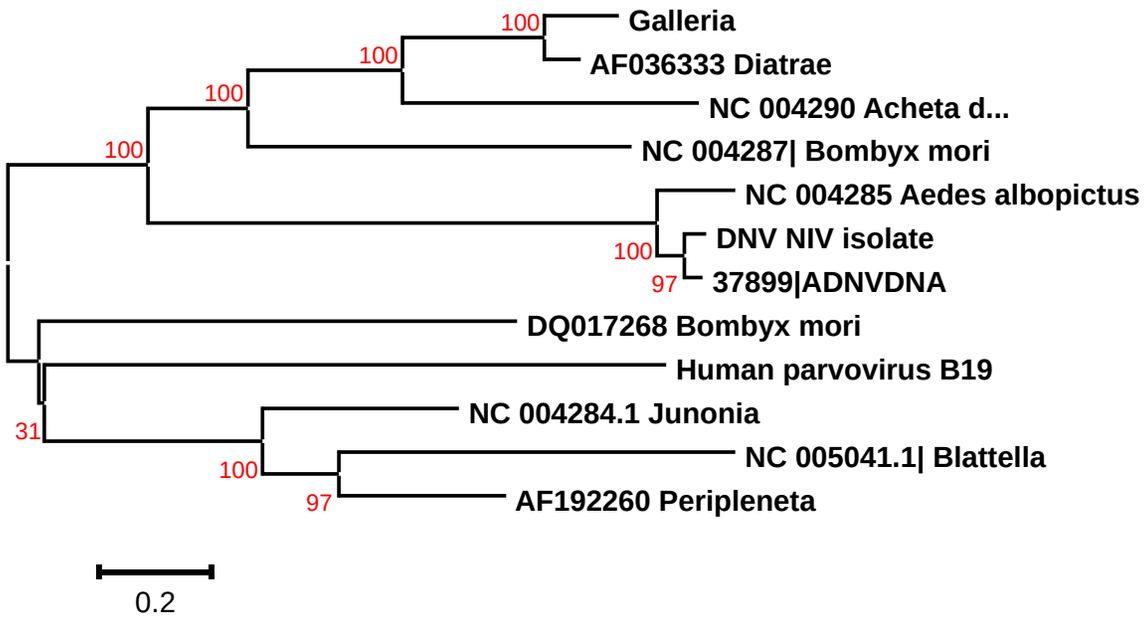


Fig. 5 : Phylogenetic analysis of complete genome of DNV viruses from different host with NIV DNV genome

Establishment and characterization of a new *Aedes aegypti* cell line

AB Sudeep, VA Arankalle and AC Mishra

A new cell line is established from *Ae. aegypti* mosquito from the neonate larvae and characterized. The epithelial like cells formed the Majority of the cell population (>90%). Seventy-six percent cells showed diploid number (2n=6) of chromosomes at the 52nd passage level. The cells showed 10-fold increase in cell number at the 52nd passage level.

Species specificity of the cell line was determined using RAPD primers **(Fig. 6)**. The RAPD profile generated using three mammalian primers *viz*. mammalian aldolase, interleukin 2í and prolactin receptor clearly demonstrated 100% homology with host insect indicating the species identity.

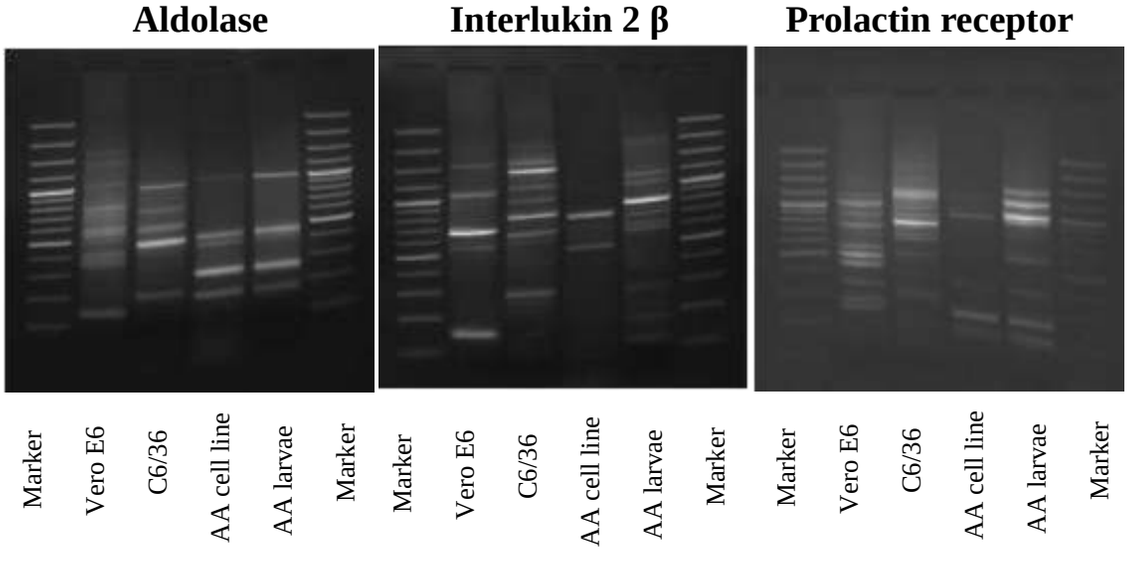


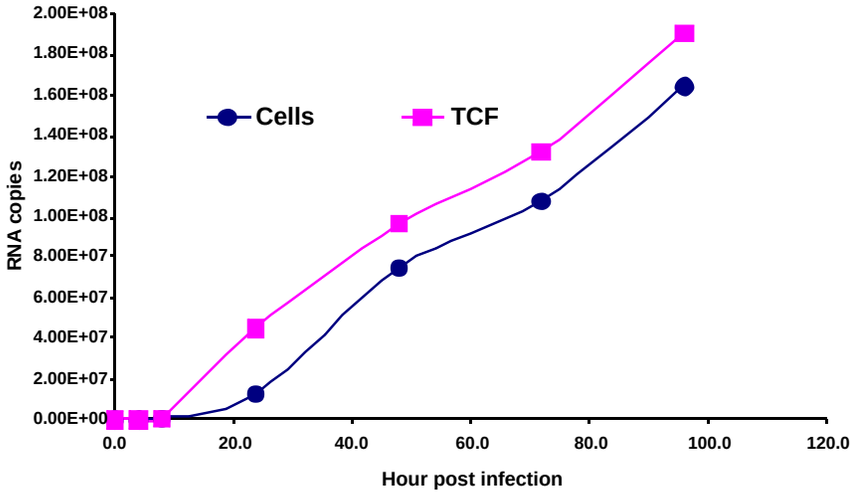
Fig. : 6

The cell line supported the replication of five mosquito-borne arboviruses (Japanese encephalitis (JE), West Nile (WN), Chikungunya (CHIK), Chittoor and dengue (DEN)) and two sandfly borne arboviruses (Chandipura and vesicular stomatitis virus). However, the cell line did not replicate two tick-borne arboviruses tested *i.e*. Ganjam and Kaisodi viruses. The virus yield of DEN, JE, WN, CHIK and Chandipura viruses in the cell line was very high and the cell line may find application in the large-scale production of antigen.

Growth kinetics of Chikungunya virus in different cell lines

Sudeep AB, Arankalle VA and Mishra AC

CHIK virus growth kinetics was studied in certain mammalian and insect cell lines to determine the virus yield. C6/36 cell line (a clone of Singh's *Ae. albopictus* cell line) was found the most sensitive and high yielding when a comparative study was carried out **(Fig. 7 & 8)**. Among the vertebrate cell lines, Vero E6 cell line yielded high titre of virus. Further studies are in progress.



Ae. aegypti cell line

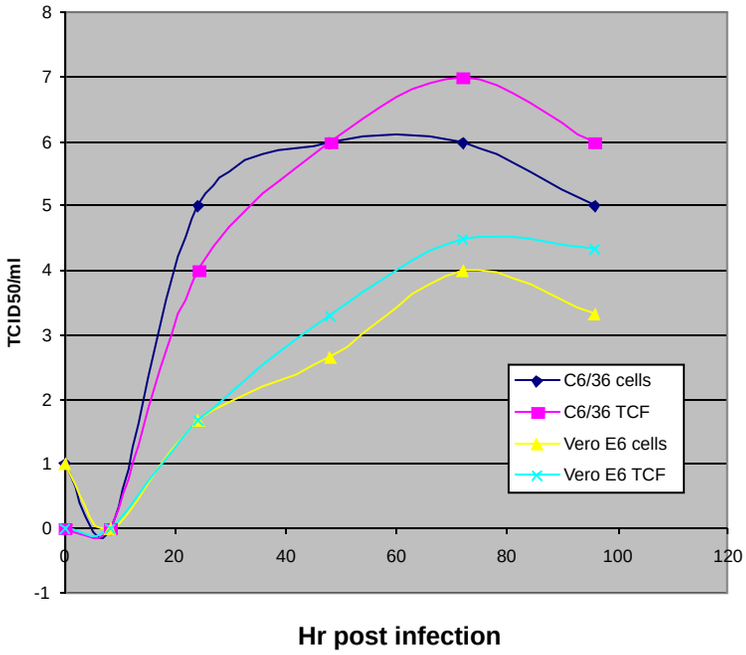


Fig. 7 & 8 : Growth of CHIK virus in certain cell lines

Epidemic investigation

Investigated CHIK virus epidemic in Kollam district, Kerala from 25th September to 5th October 2007,collected >200 human sera samples and brought to NIV Pune for further investigation**.**

Establishment of Kerala Unit at Alappuzha

Visited NIV Kerala unit, Alappuzha from 19th February 2008 to 12th March 2008 to hasten the activities of the Unit such as laboratory and lab furniture design and other related activities.

Development of an inactivated Chikungunya virus vaccine

Sudeep AB, Arankalle VA and Mishra AC

Studies are in progress for the development of an inactivated tissue culture based vaccine against Chikungunya virus. Both formalin and Beta propio lactone (BPL) inactivated virus were used to immunize mice. The inactivation kinetics of both the agents has been studied and immunogenecity is being studied **(Fig. 9)**.

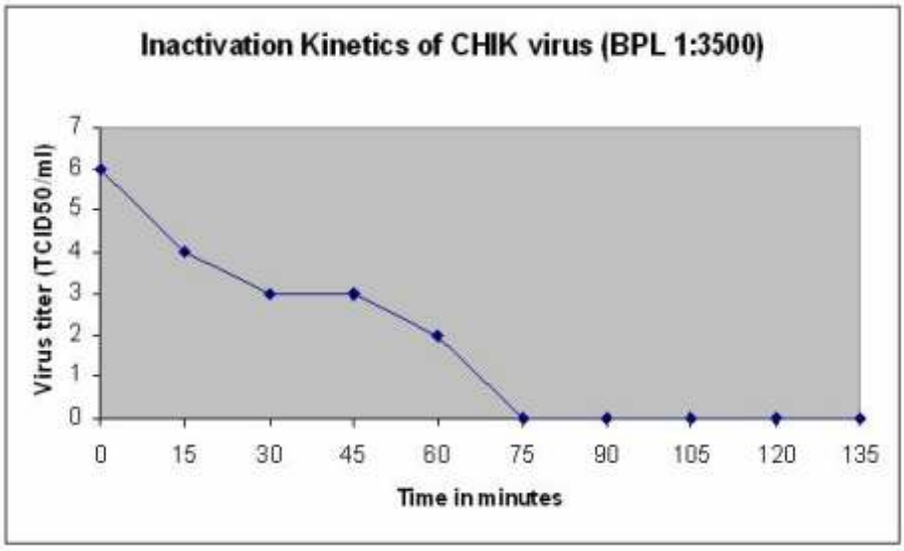


Fig. : 9

Publications

Research Articles

- Mourya DT, Gokhale MD, Kumar R. Xenodiagnosis: Use of mosquitoes for the diagnosis of arboviral infections. **J Vector Borne Dis.** 2007 Dec; 44(4):233-40.
- Yadav PD, Vincent MJ, Nichol ST. Thottapalayam virus is genetically distant to the rodent-borne hantaviruses, consistent with its isolation from the Asian house shrew (*Suncus murinus*). **Virol J.** 2007 Aug 21;4:80
- Arankalle VA, Shrivastava S, Cherian S, Gunjikar RS, Walimbe AM, Jadhav SM, et al. Genetic divergence of Chikungunya viruses in India (1963-2006) with special reference to the 2005-2006 explosive epidemic. **J Gen Virol**. 2007 Jul;88(Pt 7):1967-76.

Symposium Article

- Mourya DT, Kumar R and Gokhale MD. Bionomics of Japanese encephalitis vectors in India and control strategies. Proceedings of Eighth Sir Dorabji Tata Symposium on Arthropod Borne Viral Infections held in Bangalore on 10-11 March 2007.

Institutional documents prepared

- General Guidelines of Biosafety & Biosecurity for Institute
- Technical Manual for laboratories
- SOP for working in the laboratory

Training/workshop/symposium organized

- Conducted BSL-3 training program and a refresher course for core group on 6 and 7th June 2007.
- Preliminary training for working in High Containment Laboratory, (February 2008) conducted for NIV staff (Avian Influenza group)
- Conducted one day Bio-safety training workshop for staff and students on 24th April 2007.
- Conducted two training programs for working in High Containment Laboratory, (13-14, November 2007 and 28-29 August 2007) for State Government officials, Animal Husbandry, from Bangalore, Kolkata, Jalandar & Pune.
- Conducted one-day workshop on “Biomedical Waste Management Practices”, 29th October 2007.
- Conducted workshop on “First Aid” during Hindi Week on 14th September 2007.

Workshops / Conferences / Seminar / Meetings attended

DT Mourya

- National Conference on "Medical Preparedness and Mass Casualities Management" organized at National Disaster Management Authority during 24th and 25th July 2007 at NDMA HQrs at Hotel Centaur, Near IGI Airport New Delhi.
- Workshop on Emerging Bioagent (17-18 August, 2007), organized by DARL, DRDO, at Haldwani. Paper presented “Decontamination procedures for human pathogens during attack of bioagents”.
- Invited speaker at the Dr. Ananthanarayan Microbiology Symposium [Annual conference of Indian Association Pathology and Microbiology, 2007] at Post Graduate Institute of Medical Education and Research, Chandigarh from 27th to 29th November 2007. Title of talk “Vectors and their biology”.
- Invited speaker at the International Conference “ The advancement of science and the dilemma of dual use: Why we can't afford to fail” 9-10 November 2007, Warsaw, Poland”.
- Invited speaker at the IX International Symposium on Vectors & Vector-Borne Diseases. Puri (Orissa) India; 15-17 February 2008. Lecture on "Vectors of Japanese encephalitis in India"
- Attended symposium on ”Insecticide resistance and its management in the field populations” (14th February 2008). Puri, Orissa state.
- Invited speaker at the 56th Armed Forces Medical Conference 2008. Lecture on "Bioterrorism an Overview" at AFMC on 05 Feb 2008.
- Meeting of Research Advisory Committee (RAC) for Vector Biology and Control of National Institute on Malaria Research, Delhi held on 21st March 2008.
- Second meeting of the Expert Group constituted under the committee on Biological toxins, Weapons of Mass Destruction Act, 2005, at CCMB, Hyderabad organized by Ministry of Environment and Forests, Govt. of India, on 31st October 2007.

PV Barde

- Presented a poster titled 'Genotyping of Kyasanur Forest Disease virus strains isolated from India', at International Conference on 'Emerging and re-emerging viral diseases in the tropics and subtropics' held at IARI, New Delhi, from Dec 11-14 2007.

DR Patil

- 50th Annual Meeting of the American Biological Safety Association (ABSA) in Nashville, TN [4 10 October 2007]. During the conference training was also obtained on ABSL-3 facilities design to operations for small, medium and large animals, Effective biosafety committees and managing laboratory biosecurity program.

SRP Kumar

- Presented a poster titled "Studies on multiplication and transovarial transmission of the prototype and a recent outbreak strain of Chikungunya virus in *Aedes aegypti* mosquitoes" at International Conference on 'Emerging and re-emerging viral diseases in the tropics and subtropics' held at IARI, New Delhi, from Dec 11-14 2007.

A. Sivaram

- Presented a poster titled "Isolation & characterization of densovirus from *Aedes aegypti* mosquitoes, India" at International Conference on 'Emerging and re-emerging viral diseases in the tropics and subtropics' held at IARI, New Delhi, from Dec 11-14 2007.

Attended International Conference on emerging and re-emerging viral diseases in the tropics and subtropics held at New Delhi during 11-14 December 2007 Presented the following papers

- Sudeep AB, Jadi RS, Basu A, Arankalle VA and Mishra AC. Growth kinetics of Chikungunya virus in certain cell lines.
- Jadi RS, Sudeep AB, Arankalle VA and Mishra AC. Inactivation kinetics of Chandipura with different agents with reference to immunogenicity.

Training imparted

The following persons were trained in animal and insect cell culture.

- Mrs. Rekha Jaiswal, under WHO fellowship undergone one month training in Animal Tissue culture.
- Mr. Chetan Mokashi (M.Sc.), Modern College, Shivajinagar is undergoing a project on the characterization of insect cell lines.
- Ms. Archana Mokashe (M.Sc.), Annasaheb Magar College, Hadapsar is undergoing a project entitled; "Growth and virus yield in *Helicoverpa armigera* cell line under different nutritional and temperature conditions".
- Dr. Bharat Bhushan Sharma, Indore Biotech has undergone a short-term training course in *in vitro* cultivation of NPVs in certain lepidopteran cell lines.

Services provided

- VR and HCL staff was actively involved in recent bird flu outbreak in West Bengal.
- Laboratory diagnosis provided for Hantaan for the samples received from Apollo Hospital, Bangalore, KEM Hospital & Sassoon General Hospital.

- Quality control testing of insecticides procured for The Directorate of Health Services, (Malaria & Filaria), Government of Maharashtra, was provided.

Awards received

Dr. DT Mourya received prestigious “Bayer Environmental Science Award (2008)” for his contribution to medical entomology.

NIV Unit,
Bangalore

NIV Unit, Bangalore

Scientific Staff

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Technical Staff

Shri.Hanumaiah	Technical Officer A
Shri.R.Shanmugam	Technical Assistant B
Shri.H.Jayaprakash	Technical Assistant A
Shri.Sreerama	Technical Assistant A
Shri.M.J.Manjunatha	Technician C
Shri.M.Raju	Technician C
Shri.D.R.Shivaprasad	Technician B
Smt. B.M.Prema	Technician B
Shri.B.Thippeswamy	Technician B
Shri.N.Munivenkatappa	Technician A
Shri.H.M.Muninarayanappa	Technician A
Kum.Sharada	Technician
Kum.Shaheen Taj	Technician
Shri.Kumar Singh	Technician
Smt.Loveena D'Souza	Technician

Project Staff

Kum.Jyothi K.R.	Technician (Polio Project)ü
Kum.M.Shylaja	Technician (Polio Project)ü
Kum.T.C.Komala	Technician (Polio Project)ü
Smt.C.Ashwini	Technician (Polio Project)ü
Kum.J. Shruthi Alva	Technician (Polio Project)ü
Kum. Prithvi	Technician (Polio Project)ü

14 NIV Unit, Bangalore

- Aetiological, epidemiological and entomological studies on flavivirus infections in Karnataka and neighbouring ststes with particular reference to Japanese Encephalitis, West Nile and Dengue viruses
- Aetiological and epidemiological studies on Chikungunya virus infections in Karnataka and neighbouring states
- Studies on *Aedes aegypti* (Linnaeus) in relation to Dengue/Chikungunya virus infection in Karnataka and neighbouring States
- Surveillance of Acute Flaccid Paralysis cases from Karnataka State as a part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication Poliomyelitis Programme
- Surveillance of Measles cases from Karnataka and Kerala States, as a part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme

Aetiological, epidemiological and entomological studies on flavivirus infections in Karnataka and neighbouring states with particular reference to Japanese Encephalitis (JE), West Nile (WN) and Dengue (DEN) viruses

PN Yergolkar and Hanumaiah

Introduction

JE/WN and DEN viral infections in Karnataka and neighbouring areas have remained to be important Public Health Problems. There is continuous need to study the problem for an early diagnosis for patient management and undertaking prevention and control measures.

Objectives

- To diagnose and establish JE, WN and DEN viral infections in suspected patients in sporadic, endemic and epidemic form.
- To study epidemiological features, establish serum/virus bank and data base for prevention and control of diseases particularly JE and newly emerging Dengue Haemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS), rural and urban dengue fever outbreaks.
- To provide laboratory diagnosis to the concerned physicians for patient management and to the State and District Health authorities for undertaking necessary prevention and control measures.

Work done

Studies on JE in Karnataka State.

JE cases:

During the period a total of 5 suspected cases of encephalitis were screened for JE IgM antibodies. One 4 years female child was positive for JE IgM antibodies in serum sample from Mandikal village in Chintamani taluk of Kolar district. This case had onset in the month of March with clinical symptoms of fever, headache, vomiting, chills with weakness in all four limbs and difficulty in breathing. Other 4 cases were negative for JE IgM antibodies.

Studies on dengue (DEN) in Karnataka State and neighboring districts of Andhra Pradesh.

DEN cases:

During the period from April-2007 to March-2008 Serum Specimens were received from a total of 1971 suspected cases of DEN/CHI. Of these 1870 cases were from Karnataka and 79 cases were from neighbouring states of Andhra Pradesh (10), Tamil Nadu (2), Kerala (67), Goa (1) and Maharashtra (2) and for 22 cases address was not known.

Out of 1971 cases samples from 775 cases were tested for dengue-2 IgM antibodies by MAC-ELISA. Of these 279 (36%) cases were diagnosed as dengue in 24 districts of Karnataka and 3 positive cases were from Andhra Pradesh. For the 14 positive cases address was not known and 496 cases were negative. Details of district wise cases are presented in **Table 1.**

Table 1: District wise Diagnosis of DEN cases from Karnataka, and bordering States April-2007 to March-2008.

District	DEN IgM			No. Processed for Virus Isolation (VI)	No. Pos. for DEN VI	Total Pos. for DEN (VI + IgM)
	No. Tested	No. Pos.	No. Neg.			
Bagalkot	37	21	16	9	-	21
Bangalore Rural	49	20	29	13	-	20
Bangalore City	127	63	64	60	1	64
Bangalore Urban	12	5	7	5	-	5
Belgaum	23	12	11	61	-	12
Bidar	15	6	9	1	-	6
Bijapur	77	52	25	5	-	52
Chikaballapur	29	7	22	10	2	9
Chamarajanagar	10	0	10	10	-	0
Chikmagalur	9	0	9	3	-	0
Chitradurga	3	2	1	1	-	2
Dakshina Kannada	11	1	10	8	-	1
Davanagere	33	4	29	12	-	4
Dharwad	14	3	11	2	-	3
Gadag	4	0	4	-	-	0
Gulbarga	36	8	28	30	1	9
Hassan	45	5	40	38	-	5
Haveri	6	3	3	15	-	3
Kodagu	12	2	10	7	1	3
Kolar	32	9	23	4	-	9
Mandya	14	11	3	1	-	11
Mysore	24	3	21	30	2	5
Raichur	6	3	3	5	-	3
Ramanagara	20	8	12	11	1	9
Shimoga	41	7	34	35	1	8
Tumkur	33	6	27	10	1	7
Udupi	5	1	4	2	-	1
Karnataka Total	727	262	465	388	10	272
Andhra Pradesh	10	3	7	1	-	3
Tamil Nadu	2	0	2	1	-	0
Kerala	15	0	15	37	-	0
Address Not Known	21	14	7	7	-	14
Grand Total	**775**	**279**	**496**	**434**	**10**	**289**

Dengue activity was observed in all months **(Table 2).**

Table 2: Month wise Diagnosis of DEN cases from Karnataka and bordering States April-2007 to March-2008.

Month	DEN IgM			No. Processed for DEN Virus Isolation (VI)	No. Positive for DEN VI	Total Positive for DEN (VI + IgM)
	No. Tested	No. Pos.	No. Neg.			
April - 2007	33	16	17	35	-	16
May	42	16	26	102	1	17
June	112	31	81	64	1	32
July	37	11	26	51	3	14
August	138	58	80	57	2	60
September	96	42	54	25	1	43
October	124	58	66	29	1	59
November	69	23	46	25	-	23
December	34	9	25	10	-	9
January - 2008	21	6	15	13	-	6
February	43	6	37	28	1	7
March	26	3	23	8	-	3
Grand Total	**775**	**279**	**496**	**447**	**10**	**289**

The activity involved all age groups in both sexes. Analysis on DHF/DSS cases and dengue outbreaks in villages is being done.

Ten DEN-2 virus isolations were made in C6/36 cell line. District wise details of virus isolations are Bangalore City-1, Chikkaballapur-2, Gulbarga-1, Kodagu-1, Mysore-2, Ramnagara-1, Shimoga-1 and Tumkur-1. Monthwise details of the isolates are May-1, June-1, July-3, August-2, September-1, October- 1 and February-1 only.

In total 289 cases were diagnosed as DEN infection by MAC-ELISA and virus isolation during the period.

Summary

Low-level JE activity was recorded with 1 JE case being confirmed in Karnataka. Wide spread dengue activity was observed in Karnataka with 36.0% of the suspected cases being diagnosed as dengue in 24 out of 29 districts of Karnataka . Dengue activity was not observed in Alappuzha, Pathanamthitta, Ernakulam, Kasaragudu and Kannur districts of Kerala State during this investigation period.

Future Plan

There is continued need for surveillance, diagnosis and virus isolation for molecular epidemiology of JE and dengue infectious for undertaking preventive and control measures by the Health authorities and better management of cases by the clinicians.

Aetiological and epidemiological studies on Chikungunya (CHIK) virus infections in Karnataka and neighbouring states

PN Yergolkar, PG Jacob, PS Sathe, VA Arankalle and AC Mishra

Introduction

Chikungunya outbreaks occurred after 32 years in India and for the first time in Karnataka State in the year 2005. It was so wide spread that it affected almost all districts in one go within a period of 6 months and has added new dimension to the public health problem along with already existing dengue and JE and is still continuing to occur since then. There is need to study the pattern of CHIK infections in the coming years and monitoring of CHIK infections.

Objectives

- To diagnose CHIK infections and outbreaks for prevention and control.
- To study epidemiological features and molecular characterization of virus isolations from humans and vectors.

Work done

During the period from April-2007 to March-2008 Serum Specimens were received from a total of 1971 suspected cases of CHIK/DEN. Of these 1870 cases were from Karnataka and 79 cases were from neighbouring states of Andhra Pradesh (10), Tamil Nadu (2), Kerala (67), Goa (1) and Maharashtra (2) and for 22 cases address was not known.

A total of 521 cases were tested for Chikungunya IgM antibodies by MAC-ELISA 150 (28.8%) were positive for CHIK antibodies 371 were negative. CHIK outbreaks or sporadic cases were confirmed in 23 out of 29 districts investigated in Karnataka. One case from Madanapalli district of Andhra Pradesh and 15 cases from Kerala State were also positive. Details are presented in **(Table-3).**

Table 3 : District wise Diagnosis of CHIK cases from Karnataka and bordering States,

District	No. Processed for Virus Isolation (VI)	No. Pos. for CHIK VI	CHIK IgM		Total Positive for CHIK (VI + IgM)
			No. Tested	No. Pos.	
Bangalore Rural	13	-	29	5	5
Bangalore City	60	1	85	11	12
Bangalore Urban	5	-	9	2	2
Belgaum	61	11	24	14	25
Bidar	1	-	8	3	3
Chikbalapur	10	-	20	3	3
Chamarajanagar	10	-	10	1	1
Chikmagalur	3	-	11	1	1
Dakshina Kannada	8	-	37	20	20
Davanagere	12	-	36	3	3
Dharwad	2	1	14	2	3
Gadag	-	-	2	1	1
Gulbarga	30	1	-	-	1
Hassan	38	7	39	18	25
Haveri	15	-	14	8	8
Kodagu	7	2	19	9	11
Kolar	4	-	19	5	5
Mandya	1	-	8	4	4
Mysore	30	7	31	12	19
Ramanagara	11	3	8	1	4
Shimoga	35	-	21	1	1
Tumkur	10	1	19	4	5
Udupi	2	-	6	4	4
Uttara Kannada	11	1	2	2	3
Karnataka Total	379	35	471	134	169
Andhra Pradesh	1	-	5	1	1
Kerala	37	14	45	15	29
Grand Total	**417**	**49**	**521**	**150**	**199**

Chikungunya activity was observed in all months except March **(Table 4)** and activity involved all age groups in both sexes.

Table 4 : Month wise Diagnosis of CHIK cases tested from Karnataka, Kerala and Andhra Pradesh States.

Month	No. of Samples processed for Virus Isolation (VI)	No. Positive for CHIK Virus Isolation	CHIK IgM		Total Positive for CHIK (Virus Isolation + IgM)
			No. Tested	No. Pos.	
April - 2007	35	6	6	2	8
May	102	14	60	18	32
June	64	15	88	29	44
July	51	6	24	13	19
August	57	1	80	27	28
September	25	1	90	34	35
October	29	-	38	6	6
November	25	1	67	8	9
December	10	1	34	4	5
January - 2008	13	-	29	8	8
February	28	4	34	1	5
March	8	-	27	-	-
Total	**447**	**49**	**521**	**150**	**199**

Virus isolation

During the period a total of 445 samples were processed for virus isolation in C6/36 cell line and 49 CHIK virus isolations were confirmed by IFA and 389 samples were negative and 7 isolates are being identified. The details of isolates are Bangalore City-1, Belgaum-11, Dharwad-1, Gulbarga-1, Hassan-7, Kodagu-2, Mysore-7, Ramnagara-3, Tumkur-1, Uttara Kannada-1 and Kerala-14. Month wise isolations were April-6, May-14, June-15, July-6, August-1, Sepembert-1, November-1, December-1 and February 2008- 4. In all 199 cases were positive during the period.

Summary

Continued CHIK activity was confirmed in 23 districts of Karnataka, 3 districts of Kerala and 1 district of Andhra Pradesh during the period by laboratory confirmation of CHIK infection either by virus isolation or IgM antibody detection in 28.8% of suspected cases. Thus continuous CHIK activity since December 2005 in this region has been established indicating the endemicity established by this East Central and South African genotype of CHIKV.

Future Plan

There is continued need for surveillance, diagnosis and virus isolation for molecular epidemiology of CHIK infectious for undertaking preventive and control measures by the Health authorities and better management of cases by the clinicians.

Studies on *Aedes aegypti* (Linnaeus) in relation to DENGUE/CHIK virus infection in Karnataka and neighbouring States

PG Jacob and PN Yergolkar

Introduction

Febrile illness suspected to be due to dengue/CHIK virus infection occurred in several villages and towns of many districts of Karnataka and Kerala States between April 2007to March 2008 and entomological studies were carried out in some of these epidemic villages/towns.

Objectives

- Pattern of *Aedes aegypti* distribution and seasonal prevalence and dengue/Chikungunya virus infection.
- Factors governing the distribution of *Aedes aegypti* mosquitoes.
- Identification of important breeding sources.

Single larva survey (SLS) and *Aedes* species adult collections were carried out to find out the prevalence of the *Aedes aegypti* mosquitoes and the nature of its larval habitats. The different indices such as Breteau Index (BI number of containers positive for *Aedes aegypti* larvae for 100 houses searched), Container Index (CI denotes the percentage of containers positive for *Aedes aegypti* larvae) and Adult House Index (AHI number of houses positive for *Aedes aegypti* adults per 100 houses searched) were recorded.

Work done

i) Entomological studies in Kerala state

Entomological investigations were carried out in 4 districts, Kottayam, Pathanamthitta, Kollam and Alappuzha of Kerala state in June 2007, following reported CHIK outbreak. *Aedes* survey and adult collections were done in 17 localities (3 towns and 14 rural areas) in these districts. A total of 312 houses with 1218 occupants and a total of 3097 water holding containers, (939 from indoors and 2158 from outdoors) were surveyed. Of the total 269 positive containers 223 were with *Aedes* species larvae (13 from indoor and 210 from outdoor habitats). The three *Aedes* species recorded are *Ae. albopictus, Ae. aegypti* and *Ae. vittatus* and their proportion was 88.34, 7.17 and 4.45% respectively. The combined *Aedes* indices are as follows: HI-36.54, CI- 7.20 and BI-71.47.

Aedes aegypti mosquito breeding was recorded in three localities in Kottayam district. The BI range recorded for this species was 7.69 - 34.62.

Ae. albopictus was the predominant species in adult/immature stage collections and found breeding mainly in outdoor containers with rain water collections. This species was recorded in good numbers from latex collection cups of Rubber trees near houses, and also from the leaf axils of Pineapple and Glossina species plants.

Other mosquito species collected as immature/adults from these areas are *Anopheles subpictus, Aedes w-albus, Ae. vexans vexans, Ae.(Finlaya) pseudotaeniatus, Armigeres subalbatus, Culex gelidus, Cx. quinquefasciatus, Cx. (Lopho) minutissimus, Cx.(Lopho) uniformis, Malaya genurostris and Toxorhynchitis splendens.*

A total of 95 pools of 1678 mosquitoes of 6 species from field collected and larvae reared unfed adults of *Ae aegypti, Ae. albopictus, Ae. w-albus, Ae. vittatus, Ar. subalbatus and Cx. gelidus* were given for virus detection. Thirty one pools of *Ae. albopictus* were processed in C6/36 and/or BHK-21 cell lines and no virus has been isolated till date.

Entomological studies were also carried out in Alappuzha town of Kerala state in the month of December 2007, 67.5% of tyres with water in an old tyre dump were found positive for the breeding of *Ae. aegypti* and *Ae. albopictus* species.

Other mosquito species collected from these areas are *Ae.(Finlaya) pseudotaeniatus, Armigeressubalbatus, Culex(Eumelanomyia) brevipalpis, Heizmannia indica and Topomyia aureoventer.*

A total of 79 pools of 1682 mosquitoes (38/862 males and 41/820 females) from field collected and larvae reared unfed adults of *Ae aegypti and Ae. albopictus* were given for virus detection.

During the period in February 2008, entomological investigations were carried out Alappuzha town. Immature stages of mosquitoes were collected in outdoor cement tank and outdoor rubber tyres. *Aedes aegypti* were found in both (850 numbers collected) habitats and *Aedes albopictus* (156 numbers) was found in outdoor rubber tyres only. Pools of reared adults 21 *Ae. aegypti* and 6 *Ae. albopictus* are being process for virus isolations.

ii) Entomological studies in Karnataka state

Entomological investigations were carried out in Pushpagiri village in Mysore district and Belthangady town in Dakshina Kannada district of Karnataka state in the month of July 2007, to study the mosquito vectors involved in the suspected CHIK epidemic. *Aedes* survey and adult collections were done in these localities. *Aedes aegypti* mosquito breeding was recorded in Pusphagiri in Mysore district. The BI, HI and CI recorded for this species was 42.30, 38.46 and 3.59 respectively. *Ae. aegypti* breeding was not recorded in Belthangady where as *Ae. albopictus* was the predominant species and this species was found breeding mainly in rain water collections of outdoor containers. The indices for this species are as follows: BI- 35.19, HI- 51.85 and CI- 12.43. Other mosquito species collected along with this species from this area are *Aedes vittaus, Ae. (Finlaya) pseudotaeniatus, Armigeres auriolineatus, Ar. subalbatus, Culex quinquefasciatus, Cx. (Eumelanomyia) spp., Cx. (Lophoceraomyia) minor* and Cx*. (Lop.) uniformis.*

A total of 18 pools of 269 mosquitoes (119/9 males and 150/9 females) from field collected and larvae reared unfed adults of *Ae aegypti, Ae. albopictus* and *Ae. vittatus* were given for virus detection and are under process.

Entomological investigations were also carried out in 4 localities one each in Kodagu and Mandya districts and two localities in Bangalore Rural district in the month of October 2007. *Aedes* survey and adult collections were done in these localities. *Aedes aegypti* mosquito breeding was recorded in localities in Mandya and Bangalore` Rural districts. The BI, range recorded for this species was 13.3-35.0. In Karike village of Kodagu district *Ae. aegypti* breeding was not recorded and *Ae. albopictus* was the predominant species (BI-61.5) and this species was found breeding mainly in rain water collections of outdoor containers.

Virus Isolation

No virus could be isolated from any of the 87 pools of mosquitoes processed 38 *Ae. aegypti,* 19 each from Karnataka and Kerala and 47 pools of *Ae. albopictus* from Kerala*.* Two pools from other species were also negative for virus isolation.

Summary

Entomological survey was carried out in 5 districts of Karnataka and 4 districts of Kerala. High indices of *Aedes aegypti* and *Aedes albopictus* were shown in the outbreak localities. High proportion of *Aedes albopictus* were observed in Kerala state and Kodagu and Dakshina Kannada districts of Karnataka. No virus could be isolated from the any of mosquitoes processed for isolation from both species during this period.

Future plan

The possible role of *Aedes albopictus* and other Aedes species in the transmission of DEN/CHIK virus in the epidemic areas in Karnataka and Kerala states needs continued studies.

Surveillance of Acute Flaccid Paralysis (AFP) cases from Karnataka State as a part of WHO-SEAR Polio Lab Network in the WHO's Global Eradication Poliomyelitis Programme

PN Yergolkar and Hanumaiah

Introduction

Laboratory Surveillance work for Global Eradication of Poliomyelitis programme was initiated by the Govt. of India in collaboration with the WHO/WHO-SEARO/WHO-NPSP in India since June 1997. NIV Field Station, Bangalore is responsible for processing all the stool specimens from Karnataka State since 1997 and Kerala since September 2007 and Bareilly division of Uttar Pradesh specimens from July 2007.

Objectives

- To isolate and identify wild poliovirus from AFP cases until detection of last case of wild poliovirus in South East Asian Region and declaration of Polio free SEAR and Certification of Global Eradication of Poliomyelitis and till a global decision on discontinuation of AFP Surveillance is made.
- To participate as one of the 7 National Polio Laboratories in India in the WHO-SEAR Polio Lab Network as per guidelines of WHO's Global Polio Eradication Programme.
- To co-ordinate with State EPI Officers and National Programme Managers National Polio Surveillance Unit (Dept. of Family Welfare) and National Polio Surveillance Project (WHO) at New Delhi with weekly and monthly reports and to send all poliovirus isolates to ERC, Mumbai for intratypic differentiation tests.
- To fulfill 6 criteria of WHO for annual laboratory accreditation by the WHO to be part of the WHO-SEAR Polio Lab Network.

Work done

During the period from April 2007 to March 2008 a total of 4652 stool specimens from 2333 AFP cases were processed for virus isolation. AFP cases investigated in Karnataka were- 830, Kerala-132 and Uttar Pradesh- 1371 including other state cases investigated in Kerala and UP states. Uttar Pradesh cases investigated included 1case from Nepal, which was negative for virus isolation. Other states with AFP cases investigated in Kerala and UP were Punjab, Himachal Pradesh, Andhra Pradesh, Tamil Nadu, Goa and Maharashtra.

Wild P3 isolations were made from a total of 49 cases, of this 1 case was from Karnataka State and 48 cases were from Bareilly division of Uttar Pradesh State. Other results were Polio vaccine virus 150, NPEV- 668, Negative- 1466. Split up of 150 Polio Vaccine isolates was, P1- 77, P2- 6, P3- 53, P1+P2- 1, P1+P3- 9, P2+P3- 1 and P1+P2+P3- 3.

Wild poliovirus-3 was isolated in Karnataka from Bangalore city (Bangalore Urban district) in the month of November-2007 from a 26 months old male child. The child had migrated from Faizabad district in the month of September-2007. Isolate sequence data linked this isolate to the presently circulating wild P3 virus of Uttar

Pradesh. No secondary P3 wild cases were detected subsequently in Bangalore city or Karnataka State till the end of March-2008. This P3 wild poliovirus was isolated from an imported case in Karnataka after a gap of 3 years and 9 months. Last wild P1case in Karnataka was in February- 2004 and previous last wild P3case was in the month of December-2000.

Bareilly Division, Uttar Pradesh and Kerala AFP specimens were accepted since July and September respectively for the first time during this period in order to get NIV. Field Station, Bangalore upgraded for the Poliovirus Intratypic Differentiation (ITD) Laboratory and also to support the programme due to closure of Coonoor Laboratory and overburdening of Chennai Laboratory. Specimens were from full state of Kerala and Bareilly division of Uttar Pradesh comprised 4 districts of Bareilly, Shahajahapur, Badaun and Pilibhit. This led to the increase of workload by 3 times compared to the previous period.

Continued circulation of wild P3 virus in UP was shown by 48 positive cases from Bareilly (16), Shahajahanpur (22), Badaun (8) and Pilibhit (2) districts during the period. However no wild P1 was isolated from any of the cases during this period. Wild P3 in Uttar Pradesh was isolated in all months of the period beginning receipt of specimens since July-2007.

During the period specimens from 235 contacts of 47 index AFP cases, 4 from Karnataka and 43 from Uttar Pradesh were tested for virus isolation. No wild poliovirus was isolated from any of the contacts and 16 were polio vaccine virus, 87-NPEV only and 132 were negative.

Results of AFP cases and month wise AFP cases tested are presented in tables 5 and 6.

Table 5 : Virological results of AFP cases tested from Karnataka, Kerala States and Bareilly division cases from Uttar Pradesh.

State	AFP Cases tested	No. of samples tested	AFP Cases tested	Wild P3 virus	Polio Vaccine virus	NPEV only	Neg.
Karnataka	830	1654	830	1	33	183	613
Kerala	132	265	132	0	6	23	103
Uttar Pradesh	1371	2733	1371	48	111	462	750
Grand Total	**2333**	**4652**	**2333**	**49**	**150**	**668**	**1466**

Table 6 : Month wise results of AFP cases tested from Karnataka, Kerala States and Bareilly division cases from Uttar Pradesh.

Month	AFP Cases tested	Wild P3 virus	Polio Vaccine virus	NPEV only	Negative
April - 2007	43	0	2	11	30
May	56	0	4	19	33
June	63	0	1	21	41
July	197	2	8	75	112
August	272	9	9	122	132
September	286	10	22	65	189
October	259	10	25	57	167
November	272	5*	18	69	180
December	242	5	9	44	184
January -2008	262	2	31	57	172
February	182	1	9	51	121
March	199	5	12	77	105
Grand Total	**2333**	**49**	**150**	**668**	**1466**

* One case of P3 wild from Karnataka State.

WHO Accreditation for NIV Field Station, Bangalore in the WHO-SEAR Polio Laboratory Network

NIV Field Station, Bangalore was fully accredited by the WHO for the period. Alternate (New) Algorithm of Poliovirus isolation and identification was successfully introduced since April-2007 in the Programme.

Accreditation conditions were changed with the implementation of Alternate (New) Algorithm since January 2008. Polio Laboratory was fully accredited by the WHO for the year 2008-09. Criteria and targets achieved during 2007-08 are presented as following:

Target	Achievement
Tests are performed on at least 150 Stool specimens annually	4887 stool specimens tested.
Score on annual onsite review is at least 80%	Onsite review Score is >93%.
Test results on 80% of all AFP specimens are reported within 14 days	84% - reported within 14 days.
Accuracy of poliovirus detection in L20B isolates referred for ITD is at least 90%	93% - 328/352 polio isolates confirmed. (7%, 24/352) of isolates were NPEV's Positive in L20B)
At least 80% of poliovirus isolates from AFP cases are forwarded for ITD within 7 days	100% - 615/615 L20B positives sent within 7 days
Results on most recent Proficiency Test Panel is at least 80%	100% for March 2007 Proficiency Test

Summary

Alternate Algorithm of Poliovirus isolation and identification was successfully introduced in the programme since April-2007. Changed Accreditation conditions were introduced since January 2008. No wild poliovirus was isolated in Kerala state during the period since September-2007. Last wild poliovirus in Kerala was in the year 2000. One wild P3 was isolated from an imported case from UP in Karnataka State after a gap of 3 years 9 months. No further secondary cases were seen and the importation was successfully subdued and controlled. Continued wild P3 circulation in 4 districts of Bareilly division of UP was shown in all months since July-2007. It is important to note that no wild P1 virus was isolated during the period, which is a good indicator for the eradication programme.

Future Plan

Space has been obtained for the new proposed ITD Laboratory by upgradation of NIV, Bangalore National Polio Laboratory in the WHO-SEAR Polio Laboratory Network. Civil and electrical work are to be done for the ITD laboratory to start along with training, supply of equipments reagents undergoing proficiency test as per programme requirements.

Need to continue AFP Surveillance laboratory diagnosis as per programme requirements.

Surveillance of Measles cases from Karnataka State and Kerala State, as a part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme

PN Yergolkar and Hanumaiah

Introduction

Surveillance work for Measles cases was initiated in Karnataka by the Govt. of Karnataka in collaboration with Government of India and WHO/WHO-SEARO/WHO-NPSP in India since June 2006 along with Laboratory diagnosis of Measles and Rubella cases at NIV Field Station, Bangalore. Apart from Measles samples from Karnataka State, samples from Kerala State were also received from November-2007 onwards in view of closure of Coonoor laboratory and support for the programme so that both AFP and Measles surveillance samples from Kerala State are tested in same laboratory.

Laboratory diagnosis is carried out by detection of anti Measles IgM antibodies by using Dade Behring Measles IgM ELISA Kits. All Measles negative samples are tested for anti Rubella IgM antibodies using Dade Behring Rubella IgM ELISA kits, which are approved by the WHO and supplied for the programme. For all Measles outbreaks investigated by the programme, samples from 5 cases are collected for serology and 2 urine samples are collected for virus isolation. Measles and Rubella virus isolation is carried out in Vero/SLAM cell line supplied and approved by the programme. Measles virus is identified by Chemicon and Rubella virus by CDC IFA kits. Isolates are forwarded to the Regional Reference Laboratory (RRL) NIV, Pune for sequencing and genotyping.

Objectives

- To diagnose Measles cases by anti-Measles IgM detection by ELISA and test all Measles negative cases for Anti-Rubella IgM antibodies by ELISA.
- To isolate Measles and Rubella virus from urine samples, confirm by IFA and refer isolates for Genotyping at Global Specialized Laboratory, CDC Atlanta and also to RRL at NIV, Pune.

- To coordinate with State EPI Officers and National Programme Managers and WHO-NPSP and WHO-SEARO and to send reports of all outbreaks samples tested and also weekly report and monthly report.
- To fulfill all criteria for laboratory accreditation by the WHO to be part of the WHO-SEAR Measles Laboratory Network.

Work done

A) Laboratory diagnosis of Measles and Rubella by IgM ELISA:

During the period a total of 156 specimens from 31 outbreaks investigated were received from 14 districts of Karnataka. Of these 80 (51.3%) were positive for Measles and 76 were negative. Of the 76 negatives tested for Rubella, 17 (22.4%) were positive. Of the 31 outbreaks investigated 20(64.5%) were Measles, 6 (19.3%) were Rubella, 3(9.7%) were both Measles and Rubella and 2 (6.5%) were negative for both.

Between November-2007 to March-2008, 43 specimens from 8 outbreaks invested from 6 districts of Kerala state were tested and 21 (48.8%) were positive for Measles and 22 were negative. Of the 22 measles negatives tested for Rubella, 17(77.3%) were positive and 5 were negative for both. Of the 8 outbreaks investigated 4(50%) were Measles 2(25%) were Rubella and 2(25%) were both Measles and Rubella outbreaks. Outbreaks were investigated in the districts of Mallapuram, Kasargod, Trivendrum, Wynad, Palakkad and Kollam.

District wise serological results, Outbreak results and month wise cases tested from Karnataka and Kerala States are presented in tables 7, 8 and 9 respectively.

Table 7 : District wise Diagnosis of Measles cases (IgM positive) from Karnataka and Kerala States.

District	No. Tested	Measles	Rubella	Negative
Bangalore Urban	5	5	0	0
Belgaum	5	3	0	2
Bellary	41	16	4	21
Bijapur	5	4	1	0
Chikmagalur	5	0	5	0
Chitradurga	5	1	0	4
Davanagere	40	16	1	23
Gadag	5	5	0	0
Gulbarga	5	3	1	1
Kodagu	5	5	0	0
Koppal	10	9	0	1
Raichur	10	0	5	5
Tumkur	10	8	0	2
Uttara Kannada	5	5	0	0
Total, Karnataka	156	80	17	59
Malappuram	5	5	0	0
Kasaragodu	5	0	5	0
Trivandrum	13	6	6	1
Wynad	5	0	4	1
Palakkad	10	5	2	3
Kollam	5	5	0	0
Total, Kerala	43	21	17	5
Grand Total	**199**	**101**	**34**	**64**

Table 8 : District wise Diagnosis of Measles Outbreaks Investigated from Karnataka and Kerala States.

District	No. of Outbreaks Investigated	Outbreaks Pos. for Measles	Outbreaks Pos. for Rubella	Outbreaks Pos. for Measles and Rubella	Neg.
Bangalore Urban	1	1	0	0	0
Belgaum	1	1	0	0	0
Bellary	8	4	2	1	1
Bijapur	1	0	0	1	0
Chikmagalur	1	0	1	0	0
Chitradurga	1	1	0	0	0
Davanagere	8	6	1	0	1
Gadag	1	1	0	0	0
Gulbarga	1	0	0	1	0
Kodagu	1	1	0	0	0
Koppal	2	2	0	0	0
Raichur	2	0	2	0	0
Tumkur	2	2	0	0	0
Uttara Kannada	1	1	0	0	0
Outbreaks in Karnataka	31	20	6	3	2
Malappuram	1	1	-	-	-
Kasaragodu	1	-	1	-	-
Trivandrum	2	1	-	1	-
Wynad	1	-	1	-	-
Palakkad	2	1	-	1	-
Kollam	1	1	-	-	-
Outbreaks in Kerala	8	4	2	2	-
Grand Total	**39**	**24**	**8**	**5**	**2**

Table 9 : Month wise Diagnosis of Measles cases from Karnataka and Kerala States.

Month	Received	Measles	Rubella	Negative	No. of Outbreaks
April - 2007	15	4	0	11	3
May	5	5	0	0	1
June	5	5	0	0	1
July	5	2	0	3	1
August	5	4	0	1	1
September	10	5	5	0	2
October	15	12	0	3	3
November	15	10	0	5	3
December	20	13	5	2	4
January - 2008	30	13	5	12	6
February	10	3	4	2	3
March	64	25	15	24	12
TOTAL	**199**	**101**	**34**	**64**	**39**

B) Measles and Rubella virus isolation

During the period 42 urine samples were received from 20 outbreaks from a districts of Karnataka were processed for virus isolation in Vero/SLAM cell line. A total of 6 Measles isolates, 4 from Tumkur and 1 each from Gulbarga and Koppal districts were confirmed by Chemicon IFA. All measles isolates were sequenced at NIV, Pune Regional Reference Laboratory for Measles and N-gene sequence results indicated circulation of D8 genotype in all 3 districts. Measles outbreaks were also confirmed by IgM Serology in all these outbreaks with measles virus isolates.

During the period 13 Measles negative tissue culture fluids were tested for Rubella virus by CDC-IFA and isolation of one Rubella virus was confirmed from Chitradurga district from the outbreak of March-2007. This outbreak was negative for Measles and was positive for Rubella by IgM detection in 2 out of 5 cases tested. Rubella isolate was confirmed as Genotype2B by CDC Global Reference Laboratory. This is the first Rubella virus isolation in the WHO-SEAR Measles Laboratory Network.

Measles laboratory was fully accredited by the WHO by onsite review in May, 2007. This is the first WHO Accreditation for the Measles laboratory after functioning since June 2006.

Summary

Apart from Karnataka, laboratory surveillance for Kerala state was also undertaken. A total of 24-Measles, 8-Rubella and 5 mixed Measles and Rubella outbreaks were confirmed from both states out of the 41 Measles outbreaks investigated. Virus isolations confirmed circulating Measles genotype D8 and Rubella genotype 2B in Karnataka state.

Future Plan

Surveillance work to be continued as per Global Programme for Elimination of Measles.

Publications

- Yergolkar PN, Arankalle VA, Sathe PS, Jacob GB, Mishra AC. Chikungunya outbreaks in Karnataka and bordering states in Southern India, 2005-06. Arthropod borne Viral Infections Current Status and Research, Sir Dorabji Tata Symposium Series 2008 Volume: 8, 375-387. Edited by D. Raghunath and C. Durga Rao. Published by Tata Mc Graw-Hill Publishing Company Limited, New Delhi

Laboratory Data Contributed for following Publications:

- AFP Surveillance results for NIV, Bangalore Laboratory were published in Weekly WHO-SEAR Vaccine Preventable Disease Surveillance Bulletin, Vol. 11 and 12 from April 2007 to March 2008, Published by WHO-SEARO, New Delhi for the period.

- Measles and Rubella Serology (IgM) results, Monthly reporting, SEAR 2007-08 data for were published in Weekly WHO-SEAR Vaccine Preventable Disease Surveillance Bulletin, Vol. 11 and 12. Published by WHO-SEARO, New Delhi for the period June 2007 to March 2008.

Workshops / Conferences / Seminar / Meetings attended

PN Yergolkar

- Coordinated WHO Onsite review visit for Accreditation of Polio and Measles Laboratories in the WHO-SEAR Polio and Measles Laboratory Network at NIV Field Station, Bangalore 17-19 May 2007.
- Participated in 16th Meeting of Virologists from WHO- SEAR Polio Laboratory Network at New Delhi, 9-10 July 2007.
- Polio & Measles Lab Coordinators Meeting held by NPSU/NPSP, New Delhi at Udaipur, Rajasthan 4-5 Mar 2008.
- Attended IX - Sir Dorabji Tata Symposium on Antimicrobial Resistance held at I.I.Sc., Bangalore 10-11 Mar 2008.

Core Facilities

15 Core Facilities

- Arbovirus Diagnostics
- Electron Microscopy
- Laboratory Animals
- Library
- Maintenance
- Administration

Arbovirus Diagnostics

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Supply of MAC ELISA diagnostic kits as contribution to National Programme on monitoring incidence of JE, Dengue and Chikungunya in the country

PS Sathe

Arbovirus Diagnostics Facility :

The department of Arbovirus Diagnostics and Virus Registry is one of the service departments and has following major responsibilities

- To supply JE, Dengue and Chikungunya diagnostic kits (MAC ELISA kits) and flavivirus antigens.
- Registration of clinical samples received in the institute.
- Supply of virus strains to research organization in the country
- Screening human samples (Pre-vaccination) for HI antibodies

Supply MAC ELISA diagnostic kits and flavivirus antigens.

Eight hundred and Two MAC ELISA Kits were supplied to different organizations. Details of kits supplied is mentioned below:

JE	141
Dengue	364
Chikungunya	297
Total	802

Registration of clinical samples received in the institute.

All the samples received in the institute are registered at a central registry in the institute and given a unique identification number. During the current year, seventeen thousand three hundred and sixty seven (17367) samples were registered and issued to different department in the institute for diagnosis.

Supply of virus strains.

Seventy-three ampoules of different virus strains were supplied to various organizations and different departments within the institute. The virus strains were JE, Dengue, WN, Measles, CHIK, Herpes, CMV, and Coxsackie.

Screening human samples for HI antibodies against Flaviviruses.

Two hundred and eighty seven samples collected from people normal healthy individual (pre-vaccination) were screened for flavivirus HI antibodies. The results are mentioned in the following Table

JE	WN	Dengue	Total
P	P	P	64
N	N	N	164
P	N	N	8
N	P	N	3
N	N	P	12
P	P	N	17
P	N	P	7
N	P	P	12

P HI positive,
N HI Negative

Publications

- Mittal A, Mittal S, Bharati JM, Ramakrishnan R, Saravanan S, Sathe PS. Optic neuritis associated with chikungunya virus infection in South India **Arch Ophthalmol.** 2007 Oct; 125(10):1381-6.
- Mittal A, Mittal S, Bharathi JM, Ramakrishnan R, Sathe PS. Uveitis during outbreak of chikungunya fever **Ophthalmology,** 2007 Sep;114(9):1798
- Lalitha P, Rathinam S, Banushree K, Maheshkumar S, Vijayakumar R, Sathe PS. Ocular involvement associated with an epidemic outbreak of chikungunya virus infection. **Am J Ophthalmol** 2007 Oct; 144(4):552-6.
- Tandale BV, Tikute SS, Arankalle VA, Sathe PS, Joshi MV, Ranadive SN et al Chandipura virus: A major cause of Acute Encephalitis in children in North Telengana, Andhra Pradesh, India. **J Med Virol** 2008;80(1): 118-124.

Electron Microscopy

Scientific Staff

Dr. Atanu Basu	Scientist D	basua@icmr.org.in

Technical Staff

Mrs. SV Gangodkar	Research Assistant
Dr. Preksha Jain	Research Associate

Activities

The electron microscopy group provides a core high resolution transmission elcectron microscopy platform complete with state-of-art tissue preparation techniques to researchers within the Institute and to other National labs with due approvals. These include application areas like virus morphodiagnosis, ultrastructural review of tissues and cells, particle counts and immunoelectron microscopy. In the current year a total of 156 specimens were examined for diverse analysis. As a part of maintaining high levels of quality control, the lab is an active participant in the international EM External Quality Assurance Program co-ordinated by the Robert Koch Institute, Berlin, Germany.

Publications

- Mishra A, Panda JJ, Basu A, Chauhan VS. Nanovesicles based on self-assembly of conformationally constrained aromatic residue containing amphiphilic dipeptide **Langmuir** 2008; 24: 4571-6

Book Chapters

- Atanu B, Kanjaksha G. Dengue virus interactions with platelets. In: **Arthropod Borne viral Infections, Current status and research (Eds)** D.Rhagunath, C.Durga Rao, 2008, Tata McGraw Hill Publication, 146-151

Workshops / Conferences / Seminar / Meetings attended

- Invited talk: “Electron Microscopy of infectious diseases”, National Center for Biological Sciences, Bangalore, India, 2008
- Public lecture: “Emerging viral infections: the challenge ahead”, National Center for Biological Sciences, Bangalore India

Laboratory Animals

Scientific Staff

Dr. CG Raut	Scientist D	cgrniv@yahoo.co.in

Technical Staff

Mr. BB Gengaje	Technical Officer
Mr. SV Nipunage	Senior Technical Assistant
Mr. HL Chakankar	Technical Assistant
Mr. MP Rajarshi	Technical Assistant
Mr. SN Fulari	Technician
Mr. RH Chavan	Technician
Mr. RJ Sarpatil	Technician
Mr. SM Doke	Technician

Care, Breeding, Maintenance and Supply of Quality Laboratory Animals

Different species of laboratory animals viz. mouse; rat, hamster, guinea pig, rabbit, fowl, and geese were bred, maintained and supplied to the researchers. The number of animals from different species maintained ranged from 2387 to 2296.

A total of 3460 non SPF animals including 910 infant groups & 2099 adults of outbred Albino mice, 340 BALB/c, 83 BL/6 nude, 19 C57BL/6 mice, 4 Hamsters, 3 Guinea Pigs, 1 Rabbit and 1 Fowl were supplied to NIV scientists **(Table-1 & Fig. 1).**

Table 1: Supply of Laboratory Animals to NIV Scientists

Species	Swiss Infants	Swiss Adults	BALB/c	C57BL/6	BL/6 nude	Hamsters	Guinea Pigs	Rabbits	Fowls	Total
Numbers	910	2099	340	19	83	4	3	1	1	3460

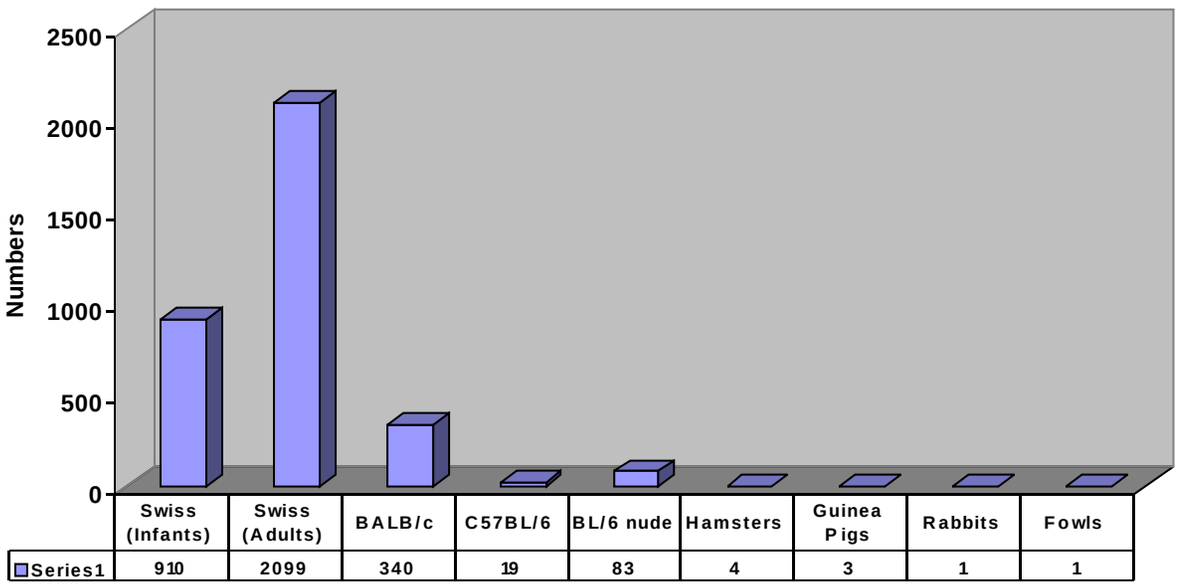


Fig. 1: Supply of Laboratory Animals to NIV Scientists

A total of 629.5 ml blood samples - 105 ml from Guinea Pigs, 150 ml Rabbits, 99.5 ml from Fowls, 188 ml from Geese and 87 ml from Sheep were supplied to NIV scientists and outside institutions **(Table - 2 & Fig. 2).**

Table 2 : Supply of Blood Samples (quantity in ml)

USER	G. PIG	RABBIT	FOWL	GEESE	SHEEP	TOTAL
TOTAL USE	105	150	99.5	188	87	629.5

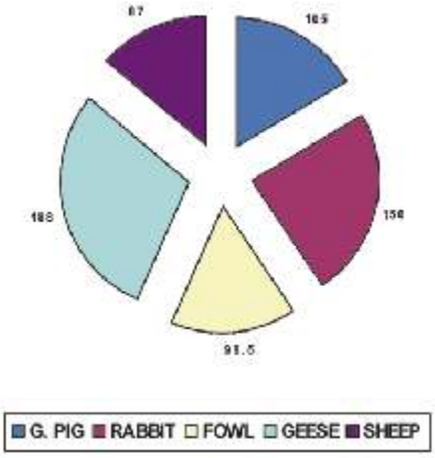


Fig. 2 : Supply of Blood Samples (quantity in ml)

Animal Health Monitoring Programme

- To have smooth functioning and defined status of animals, annual calendar of the departmental activities was developed and implemented.
- Genetic monitoring of various inbred mouse strains viz. BALB/c-AIIMS, BALB/c-Paris, BALB/c-UK, C57BL/6, C3H/HeJ, and Golden was carried out. Results obtained were within the normal limits. Deviations were observed in out bred Swiss Albino and Inbred DBA/2 strains.
- Mice were screened for various mouse pathogens like Mouse hepatitis virus, Sendai virus, Reovirus-3, Ectromelia virus, Lymphocytic Choreomeningitis Virus, rotavirus, Mycoplasma pulmonis. Some animals were found seroreactive. It is proposed to have the barrier housing systems (Individual Ventilated Cage system) for all the breeding and experimental rodent animals to have the high quality status.
- Four tumor-bearing animals were investigated for histopathological studies. Mice tumors were indicative of adenocarcinoma and rat tumours were indicative of fibroadenoma. Respective tumor samples not revealed any viral etiology by electron microscopy.
- Immunohistochemistry (IHC) was performed on intestinal sections of infants infected with rotavirus; localization of antigen was detected in epithelium of intestine.

Procurement of quality Laboratory animals

Laboratory Ferrets (*Mustela putorius*):

It is proposed to procure laboratory ferrets for the studies on influenza virus. Hence laboratory ferret suppliers were identified and contacted. Necessary permissions were obtained from the Chief Conservator of Forest, Maharashtra State, Nagpur and Expert Consultant, CPCSEA, Chennai. Initial preparation is going on for procurement, housing, care and maintenance of the ferrets.

Resource generation :

A total of 1605 surplus animals including 799 albino mice, 70 BALB/c, 38 C3H/HeJ, 50 C57BL/6, 221 NMRI nude, 131 NIH nude mice, 153 rats, 113 Hamsters and 30 Guinea Pigs were supplied on request to various research organizations registered with the CPCSEA and a sum of Rs. 2,75,505/- generated. **(Table-3 & Fig. 3)**

Table 3: Supply of Laboratory Animals to Outside Institutions

Species	Swiss Albino	BALB/c Mice	NMRI Nude	NIH Nude	C3H/ HeJ	C57 BL/6	Rats	Hamsters	G.Pigs	Total
Numbers	799	70	221	131	38	50	153	113	30	1605

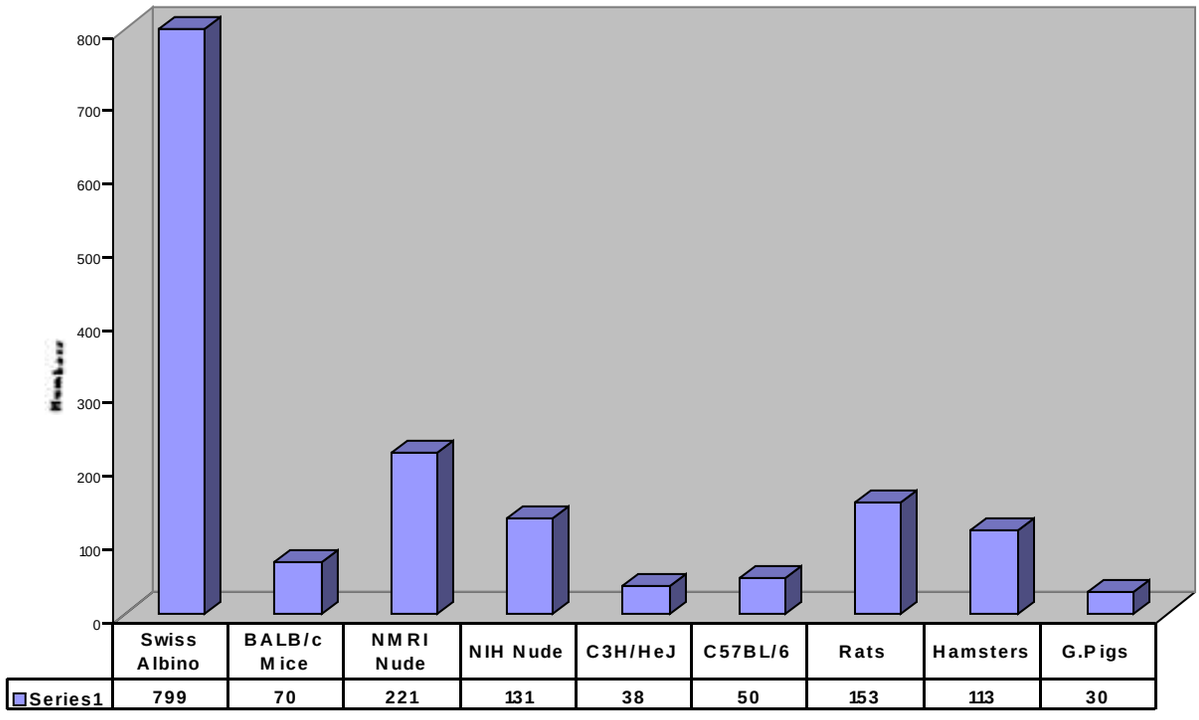


Fig. 3: Supply of Laboratory Animals to Outside Institutions

CPCSEA Activities :

- NIV Animal House facility inspected and approved by the Experts from CPCSEA, Ministry of Environment & Forests (Animal Welfare Division), Govt. of India, New Delhi
- Rehabilitation of experimental Rhesus monkeys follow-up with the Principal Chief Conservator of Forests (Wild Life), Maharashtra State, Nagpur and Chief Conservator of Forests (Wild Life), Pune Division.
- Submission of Animal Experimentation Projects for CPCSEA clearance.
- Comments/suggestions provided to the Ministry of Environment & Forests (Animal Welfare Division) & ICMR, New Delhi on the recommendations of the CPCSEA Committee on Rehabilitation of Animals after their experimentation.
- Propagation of awareness on Alternatives to lab animals: As a part of lab animal science's 3Rs principle- cell culture facility was initiated in the department. Designing & Planning of eco-friendly experimental animal facility for Sheep & Goat at Pashan. The construction work of the facility nearing to completion.
- Created ecofriendly and hygienic environment in the monkey enclosure facility.

IBSC Activities :

- Organized IBSC Meetings for grant of IBSC clearance to animal experimentation projects.
- Conducted First Aid Training programme for IBSC designated staff and distributed First Aid Kits.
- Organized Bio-safety Awareness Programme.

Management of Bio-Medical Waste :

- Preparation and submission of Annual Report of Management of Bio-medical Wastes to the MPCB authorities, Pune and Mumbai.
- A total of 7491.63 Kg. animal carcasses & other wastes have been disposed off through incineration at Central Incineration Facility of Pune Municipal Corporation.
- Incinerator Source Emission Monitoring Analysis undertaken through M/s. Horizon Services. The various incinerator parameters found to be in normal limits.

Publications

Conference /Workshop/ Proceedings Paper

- Raut CG. Classification of rodent microbes. **Workshop Book**. Workshop on microbes of rodent colony, ACTREC, Kharghar, Navi Mumbai. 2007, P10-25.
- Raut CG, Gengaje BB, Fulari SN, Nipunage SV, Waghmare SB, Zaheer SM Bacteriological assessment of conventional and barrier housing systems used for laboratory mice. **Abstract Book**- 2nd National Conference on Current Perspectives and Future Challenges in Laboratory Animal Management, IISc, Bangalore, December 29-30, 2007. p44.
- Raut CG, Rajarshi MP, Nipunage SV, Walimbe AM, Gengaje BB. Assessment of athymic nude (homozygous) mouse breeding colony for their T cell immunodeficiency. **Abstract Book**- 2nd National Conference on Current Perspectives and Future Challenges in Laboratory Animal Management, IISc, Bangalore, December 29-30, 2007. p46.
- Raut CG. Susceptibility of different laboratory animals to Chandipura virus infection by subcutaneous route. **Abstract Book**- 2nd National Conference on Current Perspectives and Future Challenges in Laboratory Animal Management, IISc, Bangalore, December 29-30, 2007. p47.
- Raut CG, Fulari SN, Gengaje BB, Nipunage SV, Deshpande AH, Raut WC. Mammary tumor occurrence in laboratory-bred mice and rats. **Abstract Book**- 2nd National Conference on Current Perspectives and Future Challenges in Laboratory Animal Management, IISc, Bangalore, December 29-30, 2007. p48.
- Senthilkumar K., Pathak VP, Raut CG, Satale SB, Brahmankar MG, Gongale MA. Pathogenesis of Indian isolate of West Nile virus in golden hamster weanlings. **Souvenir**, National Symposium on Zoonoses & Biotechnological applications, Golden Jubilee celebration, Nagpur Veterinary College, Nagpur**,** 2008.p72.
- Satale SB, Pathak VP, Raut CG, Senthilkumar K, Kale DB, Ingle SH Induced infection of West Nile virus (Indian isolate) in golden hamster sucklings. **Souvenir**, National Symposium on Zoonoses & Biotechnological applications, Golden Jubilee celebration, Nagpur Veterinary College, Nagpur**,** 2008. p73.
- Raut CG Viral Zoonoses. **Souvenir**, National Symposium on Zoonoses and Biotechnological applications, Golden Jubilee celebrations, Nagpur Veterinary College, Nagpur**,** 2008, P16-18.
- Raut CG; Thorat MG Laboratory Animal Science in India: Importance and scope of Veterinary education. **Souvenir**, National Symposium on Veterinary education in India and its impact on national economy, Golden Jubilee celebrations, Nagpur Veterinary College, Nagpur**,** 2008, P 83-85.
- Raut CG. Raut WC Importance of Laboratory Animals' Anatomy: Education, Research & Welfare. **Technical Bulletin & Souvenir**, National Seminar on "Recent advances in innovative techniques for field veterinarians in general and teaching Veterinary Anatomy in particular", KNP College of Veterinary science, Shirval**,** 2008, P9-10.

Preparation of Institutional document:

- Compendium on Laboratory Animals
- Biomedical waste management
- Technical manual for laboratory animal techniques

Workshops / Conferences / Seminar / Meetings/Training organized

- Training in "Care, Management & Experimentation of Laboratory Animals" was provided to several personnel from Veterinary, Pharmacy, Medical colleges and research institutes.
- First Aid Training Workshop was organized during Hindi Workshop for IBSC designated NIV Staff.
- Visit organized for Special Secretary to Government of Andhra Pradesh & OSD, IGCARL and other team members for establishment of Livestock Research Institute under Sri Venkateswara Veterinary University, Tirupati on 16.10.2007.
- IBSC Workshop conducted on "Bio-medical Waste Management" Guest speakers were invited from MPCB, Mumbai and Pune on 29.10.2007.
- Seminar organized on "CPCSEA guidelines", lecture delivered by Expert Consultant, Chennai at NIV/MCC, Pune on November 2, 2007.
- Training on "mice handling, immunization and bleeding" provided to research staff of NARI, Pune for the research project entitled "Generation of HIV-I subtype C based DNA vaccine candidate and assessment of prime boost immunization in mouse model".

Workshops / Conferences / Seminar / Meetings attended

CG Raut

- Participation in One Week Residential Training Programme for CPCSEA Nominees at National Institute of Animal Welfare (NIAW, Ministry of Environment & Forest, GOI), Ballabgarh 24-28 September, 2007.
- Meeting with secretarial team, Govt of Andhra Pradesh about establishing of Laboratory Animal Facility, October 16, 2007.
- Meeting related to CPCSEA inspection of lab animal facilities of NIRRH, Bombay Veterinary College & Advy chemicals, Mumbai. October 30-31,2007.
- Meeting related to CPCSEA inspection of Laboratory animal facility of Intervet India Pvt Ltd, Pune. November 2, 2007.
- Meeting at Cattle Breeding Farm, Tathwade, Pune in connection with rotavirus studies in bovines- Research project of M.Sc. (Virology) student. December 4, 2007.
- Invited speaker on the occasion of celebration of Alumni Association of KNP College of Veterinary Science, Shirval. December 12, 2007.
- Invited as a faculty for Workshop on "Microbes of Laboratory Animals" at ACTREC, Kharghar, Navi Mumbai. December 13 -15, 2007.
- Second National Conference on Current perspectives and future challenges in Laboratory Animal management. Organized by LASA, I.I.Sc. & Veterinary College Bangalore at I.I.Sc, Bangalore. Participated as co-chairman & presented papers. December 29-30, 2007.

Technical support/consultancy provided :

- Coordination of toxicological studies of legume seeds collected from encephalitis endemic area of Uttar Pradesh, conducted at Reliance life sciences, Mumbai, November 2007. Results were negative for toxicity.
- Collaborative research project on Rotavirus studies in different species of animals yielded viral isolations.
- Research support and quality services provided to the NCCS, Pune for animal experimentation.

Awards

- Received award for Hindi Poster on "Role of Laboratory Animals in Bio-medical Research", during Hindi Rastrabhashya Workshop.
- Received Best Poster Award for a poster entitled "Assessment of athymic nude (homozygous) mouse breeding colony for their T-cell immunodeficiency" by *Raut CG; et al.* presented at the Second National Conference on "Current Perspectives & Future Challenges in Laboratory Animal Management", 29-30 December 2007 at IISc, Bangalore.

Library

Staff

Dr. SN Singh (Group Leader)	Sr. Lib. & Inf. Off.	nivicl@pn3.vsnl.net.in
Mrs. VV Yewale	Asst. Lib. & Inf. Off.	vvyewale@yahoo.co.in
Mr. HM Shinde	Technical Officer	
Ms. MB Thakker	Lib. & Inf. Asst.	mbthakker@niv.co.in
Mrs. VS Chandere	Lib. & Inf. Asst.	vschandere@niv.co.in
Mr. Vishal Mali	Lib. & Inf. Asst.	vrmali@niv.co.in
Mr. SK Deshpande	Technical Assistant	

The Library has a unique and rare collection of publications in printed / digital forms in the field of viruses, viral diseases and allied subjects. It has continued to extend its information and library services not only to ICMR scientists, researchers and students but also users of DST, DBT, ICAR, CSIR, and UGC, medical and pharmaceutical organizations. NIV-Library shifted from NIV (Main Campus) to MCC (NIV), Pashan Campus. However, reference textbooks with new arrival journals and other library services continued from NIV-Ambedkar Road campus for the scientists.

Services

ERMED

ERMED **(http://nmlermed.in)** full text online IP based service added to our Library. It is a National Medical Library (NML)'s Electronic Resources in Medicine Consortium, an initiative taken by Directorate General of Health Services & Ministry of Health and Family Welfare to develop nation wide electronic information resources in the field of medicine for delivering effective health care. This has allowed the NIV-Library users to access full text 1517+ Titles of journals. This includes: Full Text- 862; NML Print Journals-655 from five publishers: ProQuest, Cambridge University Press, The Royal Society of Medicine Press, Wiley Inter Science, Lippincott Williams & Wilkins.

Science Direct

NIV-Library has subscribed for the online access of 14 core journals from Science Direct **(www.sciencedirect.com)**. This database provides full text access facility from 1996 onwards to NIV-Library users which includes total 90+ online journals exclusively for virology/viral diseases and allied subjects.

Ovid Database

NIV-Library users can also access the OVID full text database (**http://gateway.ovid.com**) for 117 online journals in the various subjects of Biomedical Research. It is fuctioned through Login & ID basis.

Other Services

- **Developing Library Network** (**DELNET)** services are added in our Library services.
- NIV-Library has also been designated as a **Nodal Centre of National Library, Kolkatta.**
- **Web OPAC LIBSYS** based services started and all the documents available are accessible to NIV- Library members.
- Automation and digitization of NIV scientific publications (papers and theses) were continued during the year: NIV **reprints- 875** and **theses- two** (Scanned) available (Total- 8) in electronic form.
- Photocopies services provided - 1,42,1345 pages
- Number of documents received on ILL - 1,712
- Number of articles/reprints received from authors - 593
- Citation Analysis of NIV Scientists' papers Provided (data five years of publication of each scientist)
- Inter library Loan, Document Delivery Services continued on demand.
- Users' awareness of NIV- Library was also provided to new comers including MSc Virology twice during the year. Current Awareness & Selective Dissemination of Information (CAS/SDI), were also continued on demand only.

New Documents Added

During the year 2007-08, the Library added the following documents and continued its services.

Books	330
Bound Volumes	218
Reports (miscellaneous) + Annual Reports	493
Reprints	586
Theses/dissertations (PhD-6; MPhil-2=8)	008
CD-ROMs	021
Journals	**Current titles of the Journals** Subscribed 079 Gratis 168 **Type of Subscribed Journals** ▪ Print + Online - 47 ▪ Print - 28 ▪ Online - 04

Work Related to Publication :

- Papers sent for publication: 21
- Papers published: 17

RTI Services

- Queries Received -29 Scientific- 3; Individuals-; CIC-3
- Queries Replied- 29 Scientific- 3; Individuals-; CIC-3

Research in Information & Library Science

Ph.D.

- Mr. Mansoor Tajdaran. A Study of knowledge skills and attitude of users to services in Academic Libraries in Tehran (Iran). (Awarded)
- Ms. Rungrudee Anusorn. Behaviour of library administrators in academic resource centres of Universities of North Eastern Thailand, using information for decision-making. (Submitted))
- Mr Ebrahim Zal Zadeh. Impact of Information Technology on Academic Departments of Library & Information Science in Iran and India: A comparative. (Submitted).
- PhD Degree awarded/submitted to PhD students: Awarded-**One**; Submitted- **Two**.

M. Phil

- M. Phil Guide Indira Gandhi Open University, New Delhi (Approved).
- M. Phil Degree awarded to M. Phil students: Awarded-**Two;** Submitted**-One**

Publications

- Singh SN, Challenges and opportunities for Library and Information Science Professionals in Digital Era. Library as a Global Information Hub: perspective and Challenges. Planner-2007, Inflibnet & Gowahati University, Gowahati 7-8th, December 2007

- Singh SN, Scientific Writing & Role of Information Officer: Challenges and Opportunities. Tenth Workshop on Medical Informatics and Biomedical Comunicatión. Bioinformatics Centre JB Tropical Disease Research Centre, MGIMS, Sevagram, Wardha, November 30 - December 1, 2007.

Conference / Workshop / Meetings Organized

Authors, Publishers and Librarians Meet 2008: Challenges in Information, Communication in Digital Era, organized by National Library of India, Kolkata and National Institute of Virology (NIV), Pune, held at NIV, Pune on January 19th, 2008.

Workshops / Conferences / Seminar / Meetings attended

SN Singh

- Libsys Training Schedule-Level-II Jayakar Library, University of Pune 18-22 February 2008
- ERMED National Library of Medicine & WHO, New Delhi 6th February 2008
- Library as a Global Information Hub: perspective and Challenges. Planner-2007, Inflibnet & Gowahati University, Gowahati 7-8th, December 2007 Paper-1
- Tenth Workshop on Medical Informatics and Biomedical Comunicación. Bioinformatics Centre JB Tropical Disease Research Centre, MGIMS, Sevagram, Wardha, November 30 - December 1, 2007.
- Selection Committee Member. National Institute of Naturopathy, Pune 23rd November, 2007 of Selection Committee for the post of Publication Officer
- Workshop on RTI. Yaswantrao Chavan Academy of Development and Administration (YASDA), Pune, 13 October, 2007.
- Selection Committee Member. National Institute of Naturopathy, Pune 16th October 2007 First Meeting of Selection Committee for the post of Publication Officer.
- Internal examiner/Guide (PhD). University of Pune, 21st September 2007.
- Examiner. Essay Writing. National Institute of Virology, Pune, 10th September 2007.
- KRM: Information Resources, Integration Access. Tata Memorial Centre, Mumbai. 3-4, August 2007.
- Editorial Board Meeting. NISAGOPCHAR VARTA Bilingual Magazine published by NIN, Pune, 13th July 2007.
- RTI for Public Information Officer. ISTM, New Delhi. 11-12, June 2007.

VV Yewale

- 2nd Level Training of LibSys Software organized by LibSys Corporation held at Jayakar Library, University of Pune, during 20-21 February 2008.
- Authors, Publishers and Librarians Meet 2008: Challenges in Information, Communication in Digital Era, organized by National Library of India, Kolkata and National Institute of Virology (NIV), Pune, held at NIV, Pune on January 19th, 2008.

VS Chandere

- 2nd Level Training of LibSys Software organized by LibSys Corporation held at Jayakar Library, University of Pune, during 20-21 February 2008.
- Authors, Publishers and Librarians Meet 2008: Challenges in Information, Communication in Digital Era, organized by National Library of India, Kolkata and National Institute of Virology (NIV), Pune, held at NIV, Pune on January 19th, 2008.

VR Mali

- 2nd Level Training of LibSys Software organized by LibSys Corporation held at Jayakar Library, University of Pune, during 20-21 February 2008.
- Authors, Publishers and Librarians Meet 2008: Challenges in Information, Communication in Digital Era, organized by National Library of India, Kolkata and National Institute of Virology (NIV), Pune, held at NIV, Pune on January 19th, 2008.
- Tenth National Convention on Knowledge, Library and Information Networking: NACLIN 2007, organized by DELNET, New Delhi, held at IIC, New Delhi during November 20-23, 2007.
- Training Programme on Biomedical Information Retrieval Programme for Medical Information Professionals/Medical Libraries organized by National Informatics Centre (NIC) New Delhi, held at NIC, New Delhi, held during July 23-27, 2007

Achievements

Dr. SN Singh has been selected as a Member of Executive Committee of Indian Association of Special Libraries and Information Centres (IASLIC) for the year 2007-09

Maintenance

Maintenance Team

S.T. Perumal	Maintenance Officer
Y.D. Sable	Asst. Maintenance Officer (Sr.)
V.M. Punekar	Technical Officer
A.R. Sable	Technical Officer
J.M. Mahadik	Senior Technical Assistant
J.D. Pacharane	Technical Assistant
N.V. Bhongale	Technical Assistant
R.M. Shukla	Technician
G.K. Bagul	Technician
N.S. Dhawale	Technician
V.D. Jagtap	Technician
B.S. Shelar	Mechanic
A.B. Kelkar	Mechanic
S.S. Holkar	Mechanic
R.S. Gadhave	Mechanic
A.M. Pawar	Mechanic
M.V. Gadhave	Mechanic

Activities

- The activities of NIV are expanding at a fast rate by way of increasing / making additions to equipments / installations every year. All essential equipments, though now are very old, are kept in working conditions.
- The services of Maintenance Staff are efficiently utilized for satisfactory operation of equipments, installations, utility services of the Institute with the available resources and without any major breakdown.
- Jobs of different nature, like preventive maintenance works for continuous operating equipments, breakdown maintenance for equipments and installations including civil/plumbing works, totaling 1052 works have been carried out.
- Major overhauling of central air-conditioning plant of Animal house has been carried out.
- Major overhauling to the cold room and freezer room machineries has been done to maintain optimum temperature out put.
- One of the imported Ultra-low temperature cabinets, which has failed in the microprocessor kit, was modified with indigenous controllers after suitable modifications and the machine is made functional satisfactorily.
- Technical support was provided for up gradation of the Influenza laboratory as per the WHO norms.
- Supports for high tech instruments and also for laboratory equipments at Bangalore Field Unit have been provided.

Administration

Mr. R. Lakshminarayanan — Senior Administrative Officer

Mr S B Attar	Administrative Officer
Mr R. V. Rao	Accounts Officer
Mr V C Khunyakari	Senior Stores Officer
Mr V V Pethe	Section Officer
Mr S S Gole	Section Officer
Mr S S Kale	Section Officer
Mr S B Pandit	Section Officer
Mr U N Shetty	Section Officer
Mr Y K Mazire	Section Officer
Mr T Shankardasan	Private Secretary
Mr P Subramanian	Private Secretary
Miss S N Ponkshe	Assistant
Mr N Shivsekhar	Assistant
Mr N G S Nair	Assistant
Mr A S Gaikwad	Assistant
Mr J S Rangan	Assistant
Mr V P Pandharkar	Assistant
Mrs S D Bhalerao	Assistant
Mr D V Kulkarni	Assistant
Mrs P K Ratnaparkhi	Assistant
Mr D T Salunke	Assistant
Mrs A S Deshpande	Assistant
Mrs A S Palshikar	Assistant
Mr H S Pasalkar	Assistant
Mrs. A S Bakare	Assistant
Mrs A Mathai	Assistant
Mrs S. Srinivasan	Assistant
Mrs V V Shendye	Assistant
Mr S E Matkar	Assistant
Mr B K Wadke	Personal Assistant
Mrs R K Amale	Personal Assistant
Mr J R Kumbhare	Personal Assistant
Mrs. Shibi Jacab	Personal Assistant
Mrs S S Dube	Stenographer
Mrs S V Adhalakha	Upper Division Clerk
Mrs A A Bapat	Upper Division Clerk
Mrs A G Ghorpade	Upper Division Clerk
Mrs S S Pathak	Upper Division Clerk
Mrs S P Mulay	Upper Division Clerk
Mrs A R Nair	Upper Division Clerk
Mrs P S Joshi	Upper Division Clerk
Mrs R S Moghe	Upper Division Clerk
Mr A D Pardeshi	Upper Division Clerk
Mr V C Chavan (Cashier)	Upper Division Clerk
Mrs S H Khamkar	Upper Division Clerk
Mrs D D Marathe	Upper Division Clerk
Mr R Basvaraju	Upper Division Clerk
Mr P N Jadhav	Upper Division Clerk
Mrs. P R Iyer	Upper Division Clerk
Mr S R Vasam	Upper Division Clerk
Mrs J V Gadre	Lower Division Clerk
Miss Jaya Jyothi J	Lower Division Clerk
Mrs P B Aher	Lower Division Clerk
Mr K S Galange	Lower Division Clerk

Training Programmes / Conferences attended

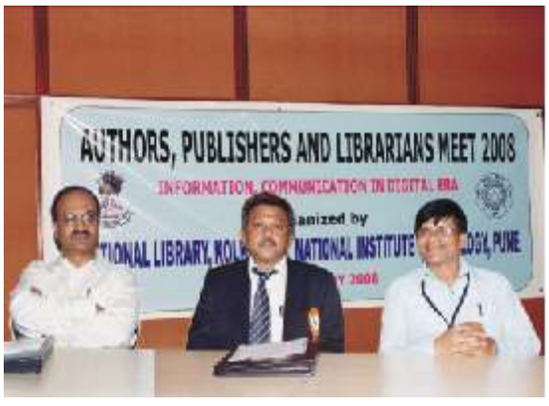
Sr. No.	Name of staff	Period of training/ Conference	Name of training/ Conference attended
1	Mr. R. Lakshminarayanan SAO	29.10.2007 to 02.11.2007	Right to Information – Trainers Development Programme. at ISTM, New Delhi.
2	Mr. SB Attar, Admn. Officer	30.07.2007 to 03.08.2007	Good Governance at ISTM, New Delhi.
3	Mr. P. Subramanian, Pvt. Secretary.	04.07.2007 to 06.07.2007	Purchase Management in Government at ISTM, New Delhi.
4	Mr. SS. Kale, SO	30.07.2007 to 03.08.2007	Good Governance, at ISTM, New Delhi.
5	Mr. YK Mazire, SO	30.07.2007 to 02.08.2007	Training Programme on Record Management, at ISTM, New Delhi.
		11.01.2008 to 13.02.2008	Training programme on Handling of CAT cases, at ISTM, New Delhi.
6	Mr. JS Rangan, Assistant	20.08.2007 to 31.08.2007	Administrative Vigilance at ISTM, New Delhi
7	Mr. SS Gole, SO	29.10.2007 to 02.11.2007	Good Governance, at ISTM, New Delhi
8	Mr. BK Wadke, Assistant.	03.12.2007 to 14.12.2007	Refresher Training Course for Personal Assistants at ISYM, New Delhi.
9	Mr. SE Matkar, UDC	08.10.2007 to 07.12.2007	Cash & Accounts course at ISTM, New Delhi.
10	Mr. AS Gaikwad, Assistant	08.10.2007 to 07.12.2007	Cash & Accounts course at ISTM, New Delhi.
11	Mrs. PK Ratnaparkhi, Assistant	21,01,2008 to 20.03.2008	Cash & Accounts course at ISTM, New Delhi.
12	Mr. PN Jadhav, UDC	21,01,2008 to 20.03.2008	Cash & Accounts course at ISTM, New Delhi.

Notable Activities

Visit of Professor Gerald T. Keusch, University of Boston, MA, USA to MCC, NIV, Pune, 17th June 2007

Visit of Shree Naresh Dayal, Health Secretary, Ministry of Health & Family Welfare, Govt. of India to NIV, Pune, 26th November 2007

Workshop on Biomedical Waste Management, 29th October 2007 : Guest faculty from Maharashtra Pollution Control Board, Mumbai



Authors, Publishers & Librarians Meet 2008, 19th January 2008

Dr.S.S.Murthy Ex.Director DESIDOC,New Delhi, Visited NIV, Pune.

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Enrichment of Infrastructure : Ecofriendly Monkey Enclosure, MCC, NIV, Pune.

M.Sc.Virology Students & Staff Second Batch