National Institute of Virology

annual report 2006

Executive Summary

The year 2006-07 was a year that defined new challenges in diverse areas of virus research like outbreak investigations, vaccine devlopement, diagnostics and gearing up state-of-art rapid response preparedness planning for possible viral pandemics of the future. Needless to say, the Institute and its dedicated crew met these challenges with professional competence and succeeded in meeting most of the set goals.

Human and avian influenza remained on a priority list. As a National Influenza Referral Center, NIV continued with the virus strain-surveillance from different geographic parts of India and molecular phylogeny studies were carried out on more than 100 virus isolates. Importantly sequence analysis for amantadine resistance carried out on 22 influenza virus isolates showed that the resistant virus strains grouped with A/Wisconsin/67/05 while the drug sensitive strains clustered with the A/New Caledonia/20/99 with 87-100 % homology. The first H5N1 avian influenza virus isolates were obtained from autopsied chicken- specimens collected during the Nawapur outbreak in Maharashtra. The complete genome of both virus isolates were sequenced and characterized. Completed genetic analysis show their sensitivity to both amantadine and oseltamivir. Presence of avian and human virulence markers like L627, E92 and v28 were also mapped in the various viral gene segments. Current studies are ongoing towards the development of better diagnostics and possible vaccine candidates using these isolate. In addition, a completely independent avian influenza group with state-of-art laboratory infrastructure embedded with the containment facility was developed along with manpower training and strong international collaboration.

Studies on the vaccine development for HEV showed promising results in mice and monkeys. The use of neutralizing-epitope within the region of the HEV ORF-2 as a candidate vaccine was noteworthy. Encapsulation of DNA and corresponding protein in liposome was found to be the best approach. Current studies are ongoing towards characterizing the full potential of this candidate vaccine. A very important research area within the hepatitis-group continued to be molecular surveillance for HAV and HEV in the environment-specially in drinking water resources. Chikungunya virus (CHIKV) pandemics continued in various parts of the

Some Memorable events of 2006-07

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International Meet on Dengue November 2006

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Visit of scientific team lead by Dr Julie Gelberding, Director,
Centers for Disease Control Atlanta, USA

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New entrance gate to the Microbial Containment Complex at Pashan

Foundation stone laying for women's hostel and
Inauguration of new hostel building

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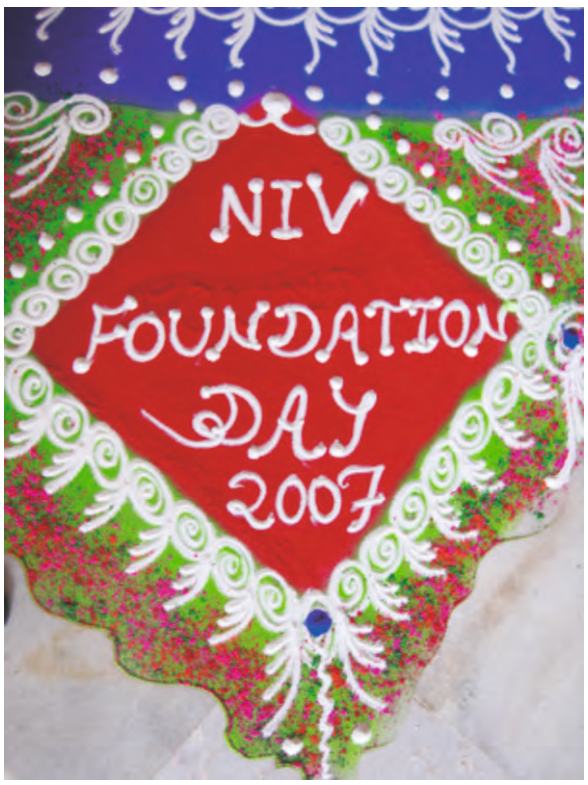

The Foundation day 2007

A very memorable landmark :
Laying of the foundation stone for the BSL4 laboratory

Miss Mitali Tadkalkar receiving the Gold Medal
for first rank holder from Prof NK Ganguly, DG ICMR

Glimpses from the Hindi Karyashala

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Outbreak Investigations

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* Other group contributions
Hepatitis
Measeles
Rotavirus
Influenza

Chikungunya virus outbreaks

Dr. B.V. Tandale

Maharashtra

In response to the request from the government of Maharashtra, NIV field team investigated the suspected Chikungunya virus (CHIKV) outbreak between 4th and 7th April 2006 in Osmanabad, Latur and Beed districts. Clinico-epidemiological details and sera were collected from 26 patients of suspected Chikungunya disease. All these were above 15 years of age and the samples comprised of 14 sera from cases reporting fever at the time of sampling and 12 more from cases without fever at sampling that had features of Chikungunya-like disease earlier. Anti CHIKV IgM was detected in 11 of 26 cases. Entomological studies included house-to-house survey for collection of adult *Aedes aegypti* mosquitoes and larvae by single larva sampling method. The observed adult household index (AHI) ranged from 10 to 30% and Breteau Index (BI) between 30% and 50%. One of 12 mosquito head squashes from Bembli village of Osmanabad district showed positivity for CHIK virus antigen by immunofluorescence test (IFA). The clinico-epidemiologic and laboratory findings implicated the Chikungunya virus as the causative agent for this outbreak. .

Gujarat

Ahmedabad city experienced Chikungunya outbreaks since May 2006. The East and Central zones were worst affected during the period. Vector indices were above critical levels. Attack rates averaged 30-40% in severely affected areas. Multiple members in the family were affected. A few hospitalizations of elderly with earlier Chikungunya illness and various systemic manifestations like neurological, renal, respiratory, cardiac and hepatic involvements were noted. There were raised levels of CPK and LDH enzymes. Routine hemograms showed leucocytosis with lymphopenia. Platelets were adequate and malarial parasites were not seen. In cases with neurological manifestations, CSF was mostly cellular with predominant lymphocytes and raised-to-normal proteins. Dengue and Leptospirosis were negative. In September 2006, investigations were carried out as a part of central team. Fifty-three patients were sampled, that included 29 cases with typical Chikungunya-like illness, 17 cases had neurological involvement and six cases showed renal, respiratory and cardiac manifestations. A total of 68 specimens were collected, which included 53 acute phase sera, 3 convalescent phase sera and 12 CSF. Thirty-four patients were confirmed. One case tested positive for anti-DEN IgM, 3 cases both for anti-CHIKV & DENV IgM and 30 were exclusively positive for anti-CHIKV IgM.In

October 2006, investigations were carried out as per request from the State Govt. Of the 61 cases investigated, 38 cases had multisystem dysfunction. Most of these were above 60 years of age. Sixty acute phase sera and 1 CSF sample were collected. The CSF sample was negative for both anti-CHIKV IgM and viral RNA. All 12 convalescent sera from these cases were positive for anti-CHIKV IgM. Anti-CHIKV IgM and RNA were detected in 45 and 11 cases respectively. In addition, 4 cases had both anti-CHIKV IgM and RNA. Confirmed CHIK infection could be established in 60 cases and CHIK viral infection was considered as the etiologic agent for the outbreaks. In addition, another 43 cases were detected from community and a total of 120 sera were collected as controls.

Suspected viral-fever outbreak with skin eruptions: Bishnupur, Manipur

Dr. MV Joshi

The Central team between 15th and 18th August 2006 investigated an outbreak of febrile eruption in Bishnupur, Manipur. The cases presented with fever of 15-30 days duration with pustular eruptions all over body in adults. Local physicians treated the patients for staphylococcal infection. These cases however were negative for staphylococci and enteric organism. Such cases were reported since 2003 with seasonality in July to September. The state team considered viral etiology in these cases. Fourteen patients were reported with 4 deaths in June & July 2006. Acute and convalescent phase sera were collected from 3 cases, acute sera from additional 2 cases and convalescent sera from 5 cases. No conclusion viral etiology could be established in these cases. All these sera were negative for JE & WN IgM antibodies.

An outbreak of Leptospirosis in Sindhudurg, Maharashtra

Dr. BV Tandale

As per State Govt. request, the investigations were done on 10th & 11th Nov. 2006. Cases presented with fever, cough, myalgia, abdominal pain and conjunctivitis. Clinicoepidemiological features suggested Leptospirosis. In some cases there was rapid onset of respiratory distress and death showing significant pulmonary bleeding on autopsy. Virological investigations for possible Hantaa virus infection were carried out at the NIV. Thirty-six cases and 6 contacts were investigated. All these cases and contacts tested negative for Hanta and anti DENV IgM. Subsequently, the State Health laboratories reported evidence of Leptospira infection in representative specimen from the outbreak using serology and molecular method.

Encephalitis outbreak, Gorakhpur

Dr MM Gore

An acute encephalitis outbreak affecting the pediatric age group was reported between April and December 2006 in Gorakhpur region of UP (India). Clinical features of the disease was characterized by moderate grade fever, hypotonia, decreased deep tendon reflexes, facial-puffiness , peripheral-edema, tachypnea, signs of congestive cardiac failure and hepato-splenomegaly in majority of the patients. Some cases presented with a Japanese encephalitis (JE)-like clinical features characterized by high-grade fever, headache, vomiting, hypertonia, meningeal irritation and altered consciousness that included seizures, convulsions and focal neurological deficit. According to the State Government Health Services, 381 deaths occurred out of 1912 reported cases (21% CFR [case fatality ratio]).

Serological and Virological and investigations

The NIV team collected 304 blood, 120 throat swabs (TS), 120 rectal swabs and 306 cerebrospinal fluid (CSF) samples from 306 representative cases admitted at the BRD Medical College & Hospital, Gorakhpur. Detailed clinical history was available from 256 patients. The specimens were primarily investigated for JE infection with Flavivirus/ JE virus specific RT-PCR, IgM ELISA and virus isolation techniques. These specimens were also investigated for Enterovirus using eneterovirus-specific RT-PCR and virus isolation. Presence of IgM antibodies against JE were detected in 36/306 CSF and 18/179 sera. Of these 14 serum-CSF pairs and only 4 single serum samples were positive. Thus a total 40 cases showed presence of JE IgM antibodies. Using nested PCR using primers from 5'NCR region 66 out of 306 CSF samples were found to be positive for entero virus RNA. Sequencing of the amplicons showed 98% homology to Enterovirus 89 and 96% homology with enterovirus 76. Six out of 66 CSF samples also showed positive sequences of Coxsackie B3 (CB3). Also all these strains showed 98% homology within the group. The average identity among Indian strain sequences of EV89/76 was found to be 99.6%.

Virus isolation

Virus isolation attempts were carried out using RD (human Rhabdomyosarcoma) and BHK (Baby Hamster Kidney) cell lines. Blind passaging of the inoculated cell cultures was carried out at least three times. Cultures showing cpe were harvested and the supernatant fluid was sujected to RT-PCR for detection of entero viral RNA and negative staining for virus-morphodiagnosis by electron microscopy (EM). Out of 10 cultures showing significant changes, 6 were from RD cells and 4 were in BHK cells. One sample that showed CPE in both RD and

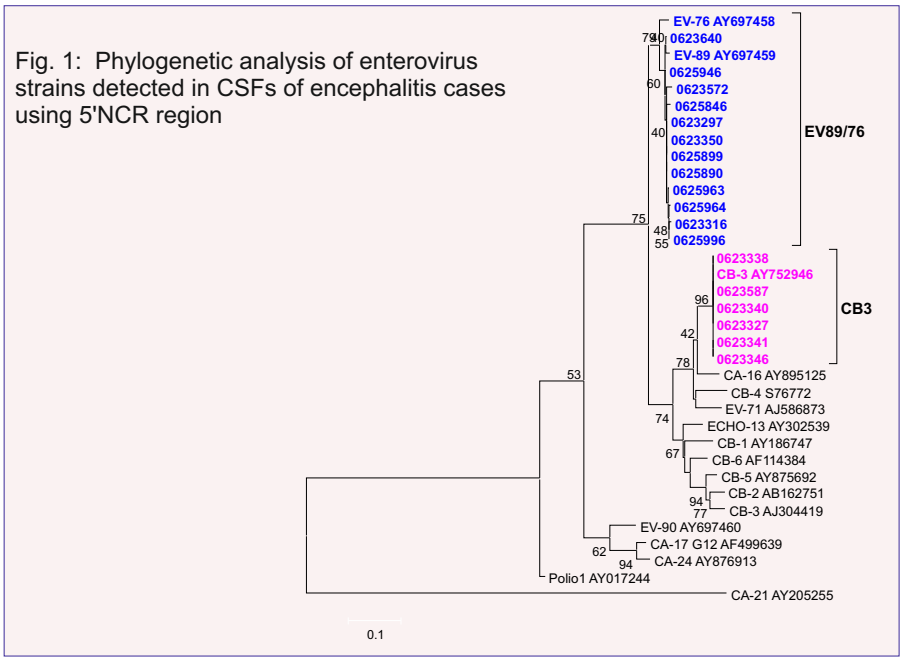

Fig. 1: Phylogenetic analysis of enterovirus strains detected in CSFs of encephalitis cases using 5'NCR region

BHK cells was characterized as a herpes virus. EM observation showed presence of 25-27 nm enterovirus-like particles of ~25nm in the culture supernatants from two CPE+ cultures. One isolate showed reactivity with the acute serum. Out of remaining 8 virus isolates, 4 isolates could be characterized as an enterovirus (Enterovirus 76/89) by RT-RCR and sequencing the amplified 5'NTR region. Further 2 isolates were also characterized as enterovirus 76 based on VP1 gene sequences. These isolates were very close to the Bangladesh isolates. Reactivity of IgM antibodies in the acute phase serum to the EV 76 isolate could be demonstrated using immunofluorescence test.

Viral Hepatitis

Dr.V.A.Arankalle

The health department of the state of Maharashtra referred sera samples representing 5 epidemics of suspected viral hepatitis to NIV for investigations. All epidemics were serologically tested and confirmed to be due to HEV.

Hepatitis E in Kedgaon

An outbreak of hepatitis was investigated at Kedgaon (Ahmednagar) in the month of Feb 2007.Total 283 cases of hepatitis were recorded in the population of 45,000 (attack rate app.1.5%) The patients were mainly adults. The first case was reported on 11th Dec.2006. Blood samples were collected from 48 patients of whom 29 were tested positive for anti HEV IgM. The results confirmed that the outbreak was due to hepatitis E virus.

Hepatitis A in Shimla

An epidemic of hepatitis was investigated at Shimla in the month of Feb 2007. The first case was detected on 21 st January 2007 and approximately 450 cases were reported up to 23 rd February. The cases were mainly noted from Kusumpti, Vikas Nagar, Panthaghati, Mehi and New Shimla locality (population around 50,000). This area receives water from Ashwini Khud water supply system. Water is collected from Ashwini Khud (natural stream), chlorinated and pumped to Kawalag storage plant and then to Kusumpti tank where it is again chlorinated and distributed to above area. Two months before the onset of the epidemic, silver ionization method was introduced for water treatment at Kusumpti tank but chlorination was re-introduced. Four kilometers upstream of Ashwini Khud water supply system; treated sewage water is let into the stream from Malayana sewage treatment plant. This sewage treatment plant is functional since one year.

Eighty-seven samples from hepatitis cases were collected either by house-to-house survey or from Reepen Hospital. Of these, 55 tested positive (71.4%) for HAV IgM while a single sample was positive for HEV IgM. The age groups were mainly children and young adults. Affluent and effluent sewage samples and treated water sample from Ashwini Khud water supply system were positive for HAV RNA by RT-PCR. The outbreak was confirmed to be due to HAV. This is the second large outbreak of hepatitis A, the first was reported by us in Kottayam, Karala.

Suspected measles outbreaks

Dr Niteen S.Wairagkar

Andhra Pradesh

(With Dr. CNS Vittal and Dr. Dr. G.V. Kumar, MeaslesNetIndia Andhra Pradesh)

Eight sporadic cases were investigated in Vijaywada. Two were investigated in Nov, 2006. Second cluster of six cases was investigated in Jan, 2007. All the cases confirmed the case definition of measles with fever and rash. Vaccination status of both cases is not known. Age group of these cases ranged from 3-4 yrs. Eight serum, 7 TS, 4 oral swabs and 5 urine samples from 8 sporadic cases, were received. All 8 sera were positive for measles IgM ELISA. All 7 T.S. were positive by RT-PCR for measles virus. The genotypes found are two D4 and five D8

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Chandigarh

(With Dr. R.K.Ratho, MeaslesNetIndia, Chandigarh)

Total 4 outbreaks were investigated from three different areas of Chandigarh namely Indira Colony (Feb. 2006), Govt. Medical College (March 06), Natwal

Haryana (April 06) and again in Indira Colony (July 06).

Indira colony outbreak: Age group of patients ranged from 5 mths to 5 yrs., in which 4 were female and 1 male, 2 cases were vaccinated, two unvaccinated and vaccination status of one was not known. Six serum, 4 TS and 2 urine samples were collected. Out of these serum samples only one was positive for measles IgM ELISA and five negative. The negative sera were tested for Rubella IgM, in which one was rubella positive.

Sporadic cases from GMC, Chandigarh: 3 cases (7 mths to 8 yrs) were investigated. Two were male and 1 female, 2 vaccinated and 1 unvaccinated. 2/3 serum samples were positive for measles IgM ELISA. Both T.S. and urine samples were negative by RT-PCR for measles virus.

Natwal, Haryana

(With Dr. R. K. Ratho, MeaslesNetindia, Chandigarh)

Thirteen cases of measles were investigated. Age group ranged from 7 mths to 1 yrs; Male:Female ratio was 4:7. Eight cases were vaccinated, 1 case was unvaccinated. Vaccination status of other cases was unknown. 12 serum, 5 TS and 4 urine samples were collected. All 12 sera were positive for measles IgM ELISA. Urine samples were processed for RT-PCR and were negative. One D8 sequence was detected from a measles case.

Indira Colony cluster of three (One serum and two T.S) cases was investigated. The serum sample was negative for Measles IgM ELISA and TS was negative by RT-PCR. The serum sample from 24 yrs female was tested for rubella and was positive for IgM antibodies to Rubella.

Tamil Nadu

(With Dr. Illankumar, Arakkonam, TamilNadu)

Measles outbreak in vaccinated older children was reported in January 2007 from a naval station, 80 kms from Chennai. The officers and sailors families live at two different places, at least 3 km apart in an enclosed campus. All the Children from the campus study in the same school. Measles cases started reporting in January till February 2007. Age group of cases ranged from 9-13 yrs. All cases had high-grade fever, cough, coryza, conjunctival congestion and macalopapular rash. Only one case had posterior cervical lymph node enlargement. Though records were not available verbal history of measles immunization obtained. None had H/o travel out of station in last two months. None had complications.Total 28 cases were detected after active surveillance in the campus area. The attack rate was 3.25%. Of these 2 cases were above 15 years of age,20 cases between 6 to 15 years (71%), 4 cases between 1-5 years and 2 cases below one year. Vaccine efficacy was estimated to be 87%. Mass

vaccination with one dose of measles vaccine was given on 20 & 21st Feb.2007. Pre and post vaccination sero-survey was done to assess the seroprevalence and seroconversion after mass immunization program. Representative samples including 10 serum samples, 6 T.S and 7 urine samples were collected during the outbreak. All three serum samples were positive for measles IgM ELISA.

Chattisgarh

(With Dr. Pravin Khobragade, UNICEF, Raipur)

There was an outbreak in Errabase Camp, Dantewada, Raipur, Chattisgarh. Age group of cases ranged from 0.6 to 7.10 yrs. M: F ratio was 10:12. Seven cases were vaccinated with measles vaccine, 8 unvaccinated while vaccination status of 7 cases was not known. Twenty One serum samples and 7 urine samples were collected. Sixteen serum samples were positive while 4 were negative for measles IgM ELISA. The urine samples were processed for RT-PCR and were negative.

Himachal Pradesh

(With Dr. Surendra Gupta, MeaslesNetIndia, Himachal Pradesh)

In September 2006 an outbreak of measles was reported in Shahpur block of Kangra district of Himachal Pradesh. The cases were found in Kotharna, Sailli, Kanol, Nauli villages of Shahpur block- Dharni-Dist Kangra. Door-to-door active case search for measles cases was initiated. About 30 cases had history &/or clinical features suggestive of measles. Seven Sera and 2 throat swabs were collected for lab investigations. Data on immunization coverage and measles vaccine efficacy, Shahpur block showed that 93.3% of children were vaccinated. Attack rate among vaccinated was 4.4% (35/790) as opposed to rate among un-vaccinated which was 28.6% (16/56). The vaccine efficacy was 84.4%.

Out of 7 serum samples, 6 were positive by IgM ELISA for Measles. Two throat swabs were processed for RNA extraction, RT-PCR and sequencing from the first set of samples received. Both the samples have shown PCR positive result, and the genotype detected is D4.

Second cluster of 6 cases was investigated in third week of November from Sarahan, Shahpur, & Hatli villages of Kangra district in third week of November 2006. These cases had clinical picture of febrile rash with conjunctivitis, cough and coryza. A large number of cases with GE/RTI complications were seen. All age group of cases was from 4-12 yrs. Six patients were found to be vaccinated. It was found that the index case came from the school game competition at Harchakiyan village of Dist Kangra. Serum, TS and urine samples were collected from each of six cases. All the serum samples were negative for measles IgM ELISA, hence were tested for Rubella IgM and four serum samples out of five

were positive. Hence the outbreak was confirmed as Rubella outbreak and the TS and Urine samples were not processed for RT-PCR for measles virus. This clearly showed that only clinical features could be misleading and laboratory investigations would only confirm the etiology.

Karnataka

(With . PN Yergolkar, NIV Field Station , Bangalore)

Several outbreaks were investigated by NPSP staff in Karnataka and specimens were referred to NIV field unit, Bangalore for serological confirmation and virus isolation. Ten TCF and 36 urine samples were referred from NIV field station, Bangalore. In all samples received the corresponding serum samples were tested for IgM ELISA at NIV Field Station, Bangalore and were all, positive. The cases belong to various districts eg. Kolar, Dhakshina Kanada, Mysore, Gadag, Bijapur, Gulburga. Age group of the cases ranged from 10 months 12 yrs. Vaccination status of these cases was not mentioned.

10 Tissue Culture Fluid from virus isolates were processed for RT-PCR and were positive. On sequencing 7 isolates belonged to D8 genotype and three were D4 genotype.

36 urine samples were processed for RT-PCR for measles virus. 4 were positive. On sequencing, 3 are D4 and one is D8 genotype of measles.Three TCF samples received from NIV field station are being processed.

Orissa

(With Dr. B.Dwibedi, RMRC, Bhubaneshawar, MeaslesNetIndia, Orissa)

Second week of October 2006 reported measles outbreak from Kuliana PHC in Orissa. Out of 26 cases, 18 are male while 8 are female. Cases ranged from 7 mths to 11 yrs of age. Only one case was unvaccinated (age < 9 mths) while all others were vaccinated for measles. Almost all cases had fever with maculopapular rash. Conjunctival congestion was observed in 12/26 cases. None had lymphadenopathy. All except two cases had neighborhood contacts while 16 had cases in family. Total 31 samples (Sera and urine from 5 cases, only serum from for 3 cases and only urine sample for 18 cases) werereceived for lab investigations. 7/7 serum samples tested were positive for measles IgM ELISA. Sequencing studies on urine samples detected two D8 genotypes.

Uttaranchal

(With Dr. Rajiv kumar, MeaslesNetIndia, Uttaranchal)

An isolated case of suspected measles was investigated in January 2007. Patient was a 6yrs male child, unvaccinated for measles and had family contact with similar case. Serum sample of this case is positive for measles IgM ELISA

and TS as well as urine samples are positive by RT-PCR for measles virus. The genotype is D8.

West Bengal

(With Dr. Prasun kumar Das, MeaslesNetIndia, Purulia, West Bengal)

An outbreak in Natandin village in Purulia district was investigated in Feb. 2006. Total 42 cases were detected during surveillance during second and third week of Feb 2006. Age group ranged from 3.5 to 13 yrs and M: F ratio 3:2. Population of outbreak area was 2200 approx. Attack rate was 1.9%. Five serum samples were referred for serological confirmation. One serum sample was positive.

Purulia

(With Dr. Dipankar Maji, MeaslesNetIndia, West Bengal)

Measles outbreak was detected in Kashipur Block, Purulia District, West Bengal, in September 2006. Door-to-door case search detected 22 cases of measles. Median age of cases was 5 years (range 3 months-16 years). No deaths occurred while 1 child developed bronchopneumonia. 68% of the total cases were vaccinated with measles vaccine. Proportion of vaccinated children in Ahartore village was 84%. The attack rate among vaccinated was 15.8% (15/95) as against 27.8% (5/18) in un-vaccinated. Vaccine efficacy was 43.2 %.Serum sample of 5 patients, Throat swab of 3 cases and Urine sample of 3 cases were collected. All cases of whom sample was collected were vaccinated. IgM antibodies for measles were detected in 4 out of 5 sera. One T.S out of three and 1 urine sample out of two were positive for measles virus RT-PCR. Both were D8 genotype.

Uttar Pradesh

(With Dr. T.N. Dhole, SGPGI, Lucknow)

In the month of January, 2007, 6 T.S., 7 urine samples and 12 serum samples from Measles cases were referred to NIV.The youngest case was of 3 yrs of age while oldest 8 yrs of age. M: F ratio is 6:11. Vaccination status of the cases is not known. All cases had occurred in 3rd and 4th week of Oct. 2006. Serology was performed at SGPGIMS and all sera tested positive for Measles IgM ELISA, hence were not tested again at NIV. T.S. and urine samples were inadequate in quantity to process of RT-PCR. Selective samples were processed directly by RT-PCR while all were inoculated in Vero slam and then TCF was used for RT-PCR. But all samples were negative on RT-PCR for Measles.

Outbreak among infants, Maharashtra

(With S. Yadav, A.Kulkarni, A.Bavdekar, Pune)

A small outbreak in infants from an Orphanage in Pune was investigated by NIV. Total seven cases were reported with classical features. Age group of cases ranged from 15 days 1 yr. Index case was a 1-year old male child who was undergoing anti-TB treatment and was hospitalized. 2/3 serum samples were positive by measles IgM ELISA. Three out of the five cases were PCR positive. The sequences obtained were all three D8 genotype. Complete H gene sequencing on 2 representative samples confirmed the genotype.

Sporadic cases

Four sporadic cases of measles were investigated in the lab. These included case of 23 years old medical student who got infected probably during his pediatric posting in the hospital wards. No other contacts had clinical features. TS were processed for RT-PCR. Two were positive out of which one was D4 and other D8. The D8 genotype was found in a medical student.

Suspected Viral Diarrhoea Outbreak

Dr.Shobha Chitambar, Dr. Gopalkrishna

An outbreak of diarrheal-illness occurred in March 2006 in southern Mumbai. Eighty eight feces specimens were collected from hospitalized cases. Eighty five fecal specimens were also obtained from asymptomatic individuals residing in the affected areas 2 months after the outbreak. All specimens were tested for Group A Rotavirus (VP6 gene), Group B rotavirus (gene5), norovirus (genogroup I and II), enterovirus (5' NCR), adenovirus (Hexon Region) and astrovirus (ORF-1a) using virus-specific polymerase chain reaction.

Group A rotaviral (but not group B) RNA was detected in 32.85% of fecal specimens . Genogroup II Norovirus RNA was detected in 12.9% specimens and enterovirus RNA was detected in 31.4% of the specimens. Adenovirus DNA was detected in 14.3% of the specimens. Co-infections with rota- and noro+rota entero + rota and adeno-viruses were also detected at (3.9%-7.8%). None of the samples was found positive for astrovirus.

Rota viral RNA was detected in 26.6% feces specimens of asymptomatic population on astro, noro and adenoviruses could be detetced. Enteroviral RNA could be detected in 6.6% of the samples. PCR products derived from representative specimens were sequenced and identity of the respective viruses was confirmed. Rotavirus VP6 gene sequence identity was >90% with simian (n=4), bovine (n=3) and human (n=11) strains. The Norovirus strains (n=9)

showed 93% nucleotide identity with that of Denmark strain. Among enterovirus positive specimens, EV-89/76(n=5), CA(n=9), EV90(n=2), Human entero mussles(n=3) and Poliovirus(n=3) were detected while the specimens positive for adenovirus DNA showed nucleotide sequences specific for type F in 7, type A in 2 and type D in one.

Suspected Hand Foot and Mouth Disease (HFMD)

Dr.Gopalkrishna V, Dr.Shobha Chitambar and PN Yergolkar

A total of 24 serum samples (12 acute phase, 12 convalescent) of patients of suspected HFMD cases were received from Malabar Institute of Medical Sciences, Calicut, Kerala. One sample of vesicular fluid from suspected HFMD case was also received from Su-Vishwas Diagnostic Lab., Nagpur. All samples were subjected to RT-PCR for the detection of enteroviruses by using primers selected from 5'NCR region (450 bp) and typing by CA-16 and EV-71 specific primers selected fromVP-1 region (251 bp). A total of six samples (five serum and one vesicular fluid) were found to be amplified in 5'NCR region. One serum sample and a vesicular fluid sample were also positive for CA-16 serotype. None of the samples were found positive for EV-71 (VP-1 gene). Phylogenetic analysis of CA-16 positive sample sequence revealed 92% homology with China and Taiwan strains available in the GenBank. None of the convalescent phase serum samples were found to be positive for enteroviruses.Thirteen cell culture isolates received from Bangalore Field Station from suspected cases of HFMD were tested for enteroviruses using RT-PCR and found negative.

Projects undertaken by Outbreak Response Group

Preparation of virus stock and immune sera for viral diagnosis

Dr. MV. Joshi

Dhori virus (NIV 611313), which had earlier undergone 3 serial passages in Swiss albinomice, was used. The pool titer was 2.2. Vero E6 cell culture was used to study the adaptation and growth of Thottapalayam (NIV 66412) virus. The virus was passaged till passage 20.Chikungunya virus (NIV 634029) infected brains were harvested for immune serum preparation.

Serosurveillance for viral infections in Andhra Pradesh

Dr. B.V. Tandale

Serosurvey for neutralization antibodies to CHPV, JEV & WNV were continued in Andhra Pradesh. In all, 237 of 291 (81.4%) sera showed presence CHPV N

antibodies and age stratification of the data showed the seroprevalence as follows: in > 15 years (206/243, 84.8%) was significantly higher than in <15 years of age (31/48, 64.6%). Only WNV N antibodies were detected in 164/ 291 (56.5%). Both JEV & WNV N antibodies were detected in 103/ 291 (35.5%) sera. The difference in N antibody seroprevalences between <15 years (11/48, 22.9) and >15 years (92/243, 38.0%) was significant. The CHPV N antibody seroprevalences in > 15 years age group was higher than the <15 years age group. JEV & WNV N antibodies were detected in 90% subjects.

Epidemologic studies on the Chandipura virus (CHP)

Dr. M.V. Joshi

Warangal, Andhra Pradesh

During 2006-07, a total of 70 cases were hospitalised, among with 21 deaths were reported with from Warangal district. Twenty-eight acute-phase sera, 22 convalescent-phase sera and 4 CSF samples were collected. All specimens from cases tested negative for CHPV RNA. An additional 99 sera were collected from contacts of encephalitis cases. All acute, convalescent and contact sera were negative for CHP IgM. JEV IgM was detected in 10/ 22 cases, 5 were having meningoencephalitis. JE cases were detected between October and December 2006 indicating seasonality.

Nagpur ,Maharashtra

Dr. B.V. Tandale

A field unit was established at the campus of the office of Deputy Director of Health Services, Nagpur Division, Nagpur. Thirty-two cases were sampled for viral investigations. 41 specimens were collected from 32 encephalitis cases, 32 acute-phase sera, 1 convalescent serum and 8 CSF. All these cases were tested negative for CHPV IgM and viral RNA. There were 4 cases with JEV IgM and 1 with exclusive WNV IgM. In addition, CHPV IgM was detected in one of 11 contacts. JE cases were detected between October and December 2006 indicating seasonality.

Publications

01. Yergolkar PN, Tandale BV, Arankalle VA et al. Chikungunya outbreaks caused by African genotype, India. *Emerg Inf Dis* 2006: 12; 1580-1583

Participation in conferences/seminars

Dr. B.V. Tandale

Oral presentation on "Seroepidemiological surveillance of acute viral encephalitis in children in Warangal, AP" in 7th APCMV, New Delhi, 13th Nov, 2006.

Poster presentation of a paper "Explosive outbreaks of Chikungunya in India after a gap of 32 years" in 7th APCMV, New Delhi, 13th November 2006.

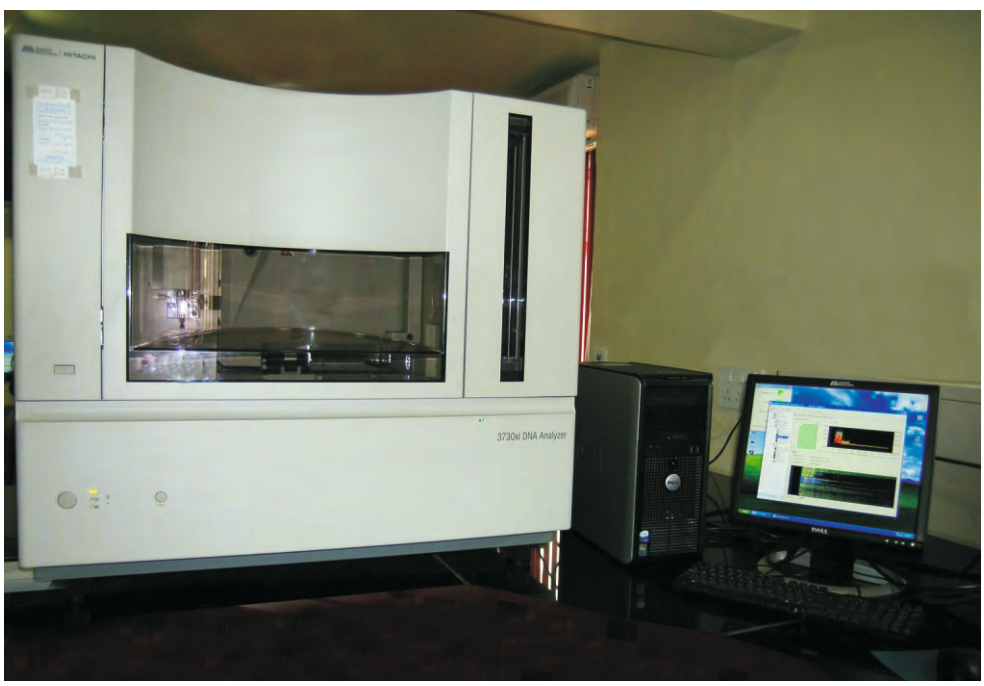
Invited lecture - "The CHIK spread" in Bone & Joint Decade National Workshop at Baramati, Pune on 15 October 2006.

Delivered a lecture on "Biosafety during field work" in the workshop on "Biosafety" for NIV technical staff held at MCC, Pashan, on 1st April 2006.

Attended National Public Health Seminar organized by School of Health Sciences, University of Pune on 2nd March 2007.

Attended Course in Clinical Epidemiology at Tata Memorial Center, Mumbai, between 5th and 9th March 2007.

Viral Hepatitis



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Viral Hepatitis Group

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Development of candidate vaccine for hepatitis E

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Hepatitis E is endemic in India and presents in epidemic as well as sporadic forms. Several large-scale epidemics are reported every year from urban as well as rural parts of India. During epidemics, there is considerable mortality among pregnant women. In sporadic settings, fulminant hepatitis E has been observed in men and non-pregnant women. Travellers to endemic areas, military personnel, sewage workers are at a high-risk of HEV infection. Hepatitis E in HBV and HCV carriers may have serious complications emphasizing the urgent public-health need for a hepatitis E vaccine.

Objectives

To develop recombinant protein-based; DNA-based and prime-boost approach based hepatitis E vaccine candidates.

Work Done

Studies in mice

This year we concluded our studies evaluating the Open Reading Frame-2 (ORF2) and the smaller region within ORF2 containing neutralizing epitopes (NE). As described earlier, these were cloned in pcDNA3.1 and pVAX1 vectors as candidate DNA vaccines and expressed in baculovirus and bacterial expression systems respectively. Humoral immune response in mice groups immunized employing different candidate vaccine approaches was determined by detecting anti-rORF2p or anti-rNEp antibodies.

Table 1 provides percent sero-conversions and comparative anti-HEV titres in mice immunized employing different approaches i.e. (1) DNA with gene gun (2) GM-CSF as a molecular adjuvant for DNA (3) recombinant protein and (4) DNA-prime-protein-boost as either 2 DNA doses and 1 protein dose (DDP) or one DNA dose and 2 protein doses (DPP). Both ORF2 and NE proteins alone were less immunogenic, geometric mean anti-HEV titres being 9.8 ± 1.6 and 4.0 ± 1.4 respectively whereas in the DNA alone format both regions were found to be better immunogens, ORF2 (GMT=855.6± 1.5) being superior to NE (GMT=40.0 ± 1.6). However, when the mice were immunized with two DNA and one corresponding protein dose at 0, 4, 8 weeks, the boosting effect was prominent with NE region (GMT=174.0 ± 3.0), while titres with ORF2 (487.4 ± 2.2) remained comparable to 3 ORF2 DNA doses (833.6 ±1.3).

DNA-prime-protein-boost

Swiss albino mice (n=8) were primed with 1.0µg of ORF2 DNA and subsequently boosted with 2.0µg of rORF2p in 2 different dose schedules (short and long) and 2 different dose regimens (DDP and DPP) in DNA-prime-protein-boost approach. Seroconversion rates in these mice groups are given in Table 1. HEV-specific antibody titers of Swiss albino mice groups immunized employing different ORF2 based approaches are showed in Figure 1. The reciprocal specific antibody titers in ORF2-S-DDP, ORF2-S-DPP, ORF2-L-DDP and ORF2-L-DPP groups ranged between 40 to 6400, 160 to 3200, 1280 to 12800 and 320 to 12800 respectively with two mice remaining non-responders in the last group. The specific antibody titers in ORF2-L-DDP group were significantly higher than ORF2 DNA group ($P<0.05$) while in the remaining three groups the antibody titers did not differ significantly.

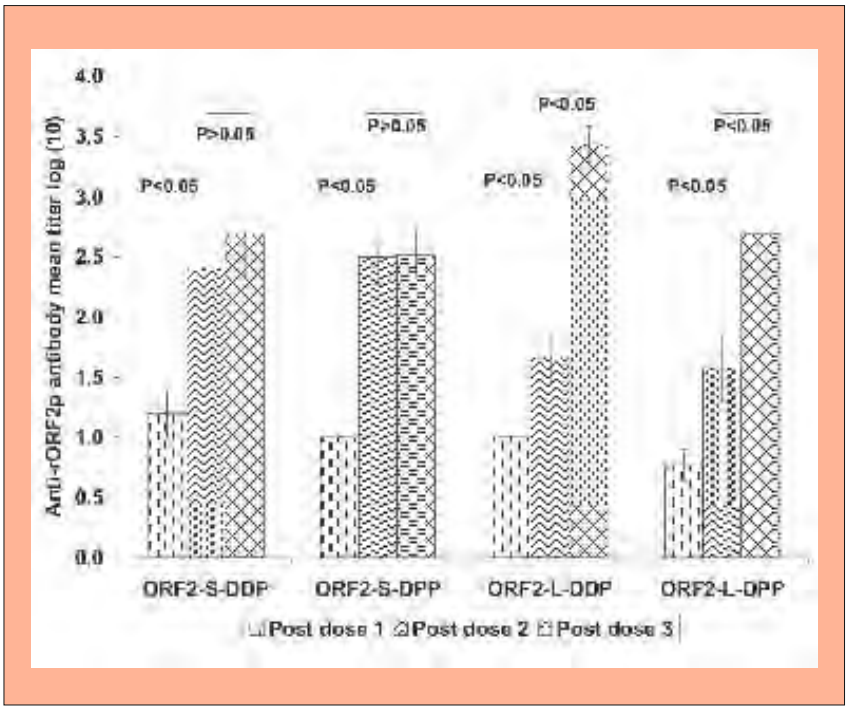

Figure 1: Serum HEV-specific anti-rORF2p IgG antibody mean log (10) titers detected in ELISA just before dose 2 and 3, and 2-3 weeks post dose 3 in Swiss albino mice groups immunized with ORF2 DNA and protein in DDP and DPP regimens of both short (0, 4, 8 weeks) and long (0, 4, 20 weeks) schedules (total 3 doses, 1µg/dose/plasmid of ORF2 DNA and 2µg/dose of rORF2p). Error bars represent standard error of the mean log titer.

Figure 2 depicts dose-wise anti-HEV response in different groups of mice. The specific antibody titer increased after every dose (total 3 doses) in ORF2-S-DDP, ORF2-L-DDP and ORF2-L-DPP groups. The antibody titer after dose 3 (last dose) increased by ~2-folds in mice immunized in DDP regimen of short interval dose schedule (ORF2-S) whereas titers increased by ~60-folds and ~13-folds after 3rd dose (last dose) in mice immunized in DDP and DPP regimens respectively in long interval dose schedule (ORF2-L).

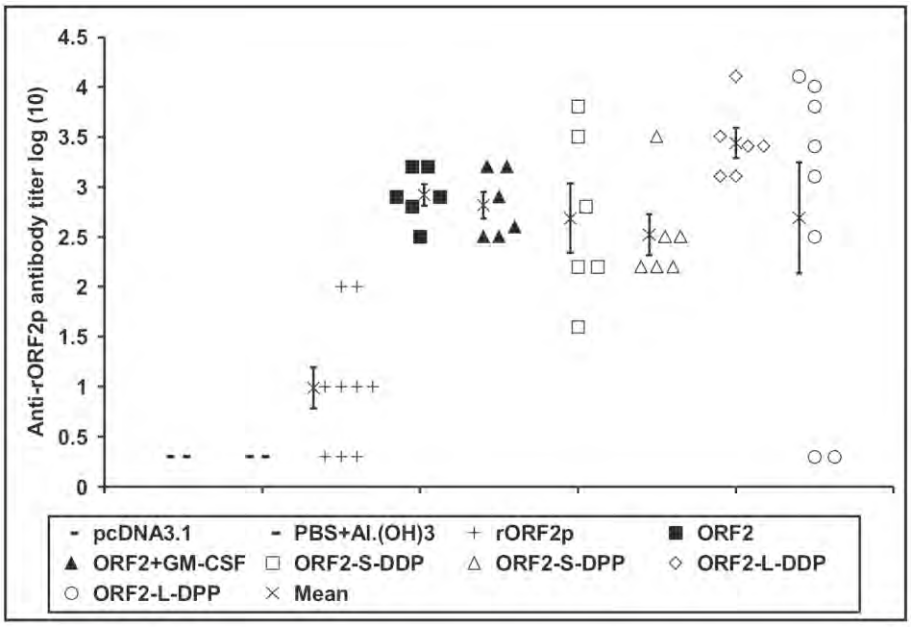

Figure 2: Serum HEV-specific anti-rORF2p IgG antibody log (10) titers detected in ELISA at 2-3 weeks post last dose (3rd dose) in Swiss albino mice groups immunized (total 3 doses, 1μg/dose/plasmid DNA and 2μg/dose of rORF2p) with rORF2p (0, 4, 8 weeks), ORF2 DNA (0, 4, 8 weeks), ORF2 DNA with GM-CSF DNA (0, 4, 8 weeks), ORF2 DNA and protein in DDP and DPP regimens of both short (0, 4, 8 weeks) and long (0, 4, 20 weeks) schedules. Error bars represent standard error of the mean log titer.

The seroconversion rates in Swiss albino mice (n=10) primed with 1.0μg of NE DNA and subsequently boosted with 2.0μg of rNEp in DDP dose regimen in short interval dose schedule (0, 4 and 8 weeks; NE-S) are given in Table 1. The reciprocal specific antibody titers in this group ranged from 400 to 3200 with 2 mice remaining non-responders. The geometric mean HEV-specific antibody titer (174.0±3.0) in these mice was higher than those observed in mice immunized with total 3 doses of NE DNA alone (40.0±1.6), NE DNA in combination with GM-CSF DNA (43.3±2.7) and rNEp alone (4.0±1.4) (Tabal 1).

Table 1: Seroconversion rates and HEV-specific antibody titers in mice groups immunized employing different approaches

aMice (n)[d]	Dose and dose schedule	Percent seroconversion			Geometric mean titer±SEM (n)[e]
		Post dose 1[a]	Post dose 2[b]	Post dose 3[c]	
Balb/c (n=10)	ORF2 DNA, 0, 2, 4 weeks	0%	20%	100%	158.4 ± 1.9 (n=10)
"	ORF2 and GM-CSF DNA, 0, 2, 4 weeks	0%	40%	100%	268.2 ± 1.9 (n=7)
"	ORF2 DNA, 0, 4, 8 weeks	0%	10%	20%	5.5 ± 1.9 (n=10)
"	ORF2 and GM-CSF DNA, 0, 4, 8 weeks	0%	0%	50%	7.1 ± 1.6 (n=10)
Swiss albino (n=11)	ORF2 DNA, 0, 4, 8 weeks	0%	90%	100%	833.6 ± 1.3 (n=6)
"	ORF2 and GM-CSF DNA, 0, 4, 8 weeks	18%	100%	100%	661.6 ± 1.4 (n=10)
Swiss albino (n=10)	rORF2p, 2µg/dose 0, 4, 8 weeks	0%	20%	77.7%	9.8 ± 1.6 (n=9)
Swiss albino (n=8)	ORF2-S-DDP 0, 4, 8 weeks;	100%	100%	100%	487.4 ± 2.2 (n=6)
"	ORF2-S-DPP 0, 4, 8 weeks;	100%	100%	100%	332.1 ± 1.6 (n=6)
"	ORF2-L-DDP 0, 4, 20 weeks,	100%	100%	100%	2757.6 ± 1.4 (n=6)
"	ORF2-L-DPP 0, 4, 20 weeks;	62.5%	62.5%	62.5%	493.3 ± 3.6 (n=8)
Swiss albino (n=6)	NE DNA, 0, 4, 8 weeks	0%	17%	100%	40.0 ± 1.6 (n=4)
"	NE and GM-CSF DNA, 0, 4, 8 weeks	0%	0%	67%	43.3 ± 2.7 (n=4)
Swiss albino (n=7)	rNEp, 0, 4, 8 weeks	0%	14.2%	28.6%	4.0 ± 1.4 (n=7)
Swiss albino (n=10)	NE-S-DDP 0, 4, 8 weeks;	0%	0%	60%	174.0 ± 3.0 (n=8)

a Percent seroconversion at 2 or 4 weeks post dose 1.

b Percent seroconversion at 2 or 4 or 16 weeks post dose 2.

c Percent seroconversion at 1 week after the 3rd dose (last dose).

d Number of mice taken in each group.

e HEV-specific IgG antibody geometric mean titers determined at 2 to 3 weeks post last dose (3rd dose) for immunized mice groups. Number in parentheses denotes the number of mice taken from each group for titer determination.

ELISA based in-vitro HEV binding/neutralization assay

In the absence of availability of an *in-vitro* or *in-vivo* neutralization assay for the detection/titration of anti-HEV neutralizing antibodies in the immunized mice, an ELISA based virus binding/neutralization assay was standardized. Pre and post-inoculation serum samples from a rhesus monkey experimentally infected with HEV and a 10% (w/v) stool suspension containing $1.3X10^7$ RNA copies of HEV/ml were used as the antibody and virus standards respectively. In rORF2p based ELISA, the pre-serum was scored negative whereas the HEV-specific antibody titer of the post-infection serum was 1:6400. This serum was diluted 2-folds serially, and equal volumes of the diluted sera were allowed to react with the virus positive stool suspension at 37°C for 1 hour. Presence of specific antibody in the serum-virus mixture was assessed in ELISA as described earlier. Samples showing > 50% reduction in OD values in ELISA when compared with the respective control (serum + PBS) were considered positive for virus-binding/neutralizing antibodies (Table 2). Following standardization of the assay, pre and post-immunization serum samples from mice immunized employing different protocols were similarly assessed for the presence of virus binding/neutralizing antibodies (Table 2).

Table 2: Binding/neutralization of HEV with anti-HEV antibodies in a monkey experimentally infected with the virus and mice immunized with vaccine candidates

Species and Details	Serum dilution	OD values (492nm) in rORF2p based ELISA	
		Serum+PBS	Serum+Virus
Rhesus monkey-Post-inoculation	1:100	1.738	1.213
	1:200	1.778	0.708
	1:400	1.521	0.372
	1:800	1.151	0.085
	1:1600	0.634	0.055
	1:3200	0.475	0.06
	1:6400	0.195	0.022
Rhesus monkey-Pre-inoculation	1:100	0.083	0.099
Mice immunized with			
ORF2 DNA	1:100	0.711	0.075
ORF2 DNA+GM-CSF DNA	1:80	0.800	0.122
ORF2-S-DDP	1:400	0.914	0.021
ORF2-L-DPP	1:200	0.977	0.081
NE DNA	1:20	0.249	0.107
NE DNA+GM-CSF DNA	1:100	0.447	0.045
NE-S-DDP	1:200	0.826	0.092
Pre-immune	1:10	0.073	0.077

Cell mediated immune response

Results of the LPA are depicted in Tables 3 and 4. Splenocytes from control group mice of both strains did not respond to both the recombinant antigens (SI value range 0.5 to 2.4 with rORF2p and 0.008 to 2.6 with rNEp). The SI values in PHA stimulated cells ranged from 0.3 to 132.3. The SI values in the immunized Swiss albino mice groups are shown in Table 4. Percent recall response was less in Balb/c mice compared to Swiss albino mice. Thus, immunization with ORF2 DNA/rORF2p and NE DNA/rNEp based approaches induced cell mediated immune response in Swiss albino mice.

Table 3: The stimulation indices (SI) in immunized Swiss albino mice groups as assessed by LPA

	SI value range, median value, P value	
	Recall antigen	
	rORF2p	**rNEp**
ORF2 DNA	3.0 to 6.1, 3.0, P < 0.01	ND[a]
ORF2 DNA+GM-CSF DNA*	0.03 to 3.8, 1.5, P > 0.05	ND[a]
rORF2p	0.9 to 5.4, 1.7, P > 0.05	1.0 to 7.2, 1.5, P > 0.05
ORF2-S-DDP[b]	0.3 to 4.6, 1.9, P = 0.051	0.9 to 6.0, 1.7, P < 0.01
ORF2-S-DPP[b]	1.9 to 10.5, 3.3, P < 0.01	2.2 to 4.9, 2.6, P < 0.01
NE DNA	1.4 to 4.5, 2.6, P < 0.01	1.2 to 6.1, 3.4, P < 0.01
NE-S-DDP[b]	0.2 to 19.4, 7.5, P < 0.05	1.2 to 16.9, 6.7, P < 0.01

* Mice co-immunized with 1.0µg/dose of GM-CSF DNA
a Not done
b DNA prime protein boost

Table 4: Cell-mediated immune response in Swiss albino and Balb/c mice

Approach[c]	**Responder/Total tested (% Recall response)**							
	Swiss albino				**Balb/c**			
	Control mice		**Test mice**		**Control mice**		**Test mice**	
	Recall antigen		**Recall antigen**		**Recall antigen**		**Recall antigen**	
	rORF2p	rNEp	rORF2p	rNEp	rORF2p	rNEp	rORF2p	rNEp
ORF2 DNA	0/5 (0%)	0/5 (0%)	6/11 (54.5%)	ND[a]	0/5 (0%)	ND[a]	1/12 (8.3%)	ND[a]
ORF2+GM-CSF DNA*	0/5 (0%)	0/5 (0%)	2/11 (18.2%)	ND[a]	0/5 (0%)	ND[a]	0/14 (0%)	ND[a]
rORF2p	0/5 (0%)	0/5 (0%)	3/9 (33.3%)	4/9 (44.4%)	ND[a]	ND[a]	ND[a]	ND[a]
ORF2-S-DDP[b]	0/5 (0%)	0/5 (0%)	2/7 (28.6%)	2/7 (28.6%)	0/5 (0%)	ND[a]	ND[a]	ND[a]
ORF2-S-DPP[b]	0/5 (0%)	0/5 (0%)	3/6 (50%)	2/5 (40%)	0/5 (0%)	ND[a]	ND[a]	ND[a]
NE DNA	0/5 (0%)	0/5 (0%)	3/9 (33.3%)	5/9 (55.6%)	0/5 (0%)	ND[a]	ND[a]	ND[a]
NE-S-DDP[b]	0/5 (0%)	0/5 (0%)	7/8 87.5%	6/8 75.0%	ND[a]	ND[a]	ND[a]	ND[a]

* Mice co-immunized with 1.0µg/dose of GM-CSF DNA
a Not done
b DNA prime protein boost
c All DNA doses were given by gene gun and protein by needle-injection.

Anti-HEV IgG isotype analysis

Anti-HEV antibodies in the mice sera were reactive to all the IgG subclasses (1, 2a, 2b and 3) (data not shown). Since, IgG3 isotype was low in all the groups, titers for IgG1, 2a and 2b were determined for Swiss albino mice. The IgG2b titers were significantly lower than the 1 and 2a in all the mice groups (data not shown). For mice immunized employing different approaches the predominant isotype detected was IgG1 . Pre-immune and control mice sera were non-reactive for the HEV-specific IgG and all its isotypes. This IgG1 predominance is suggestive of Th2 type of immune response.

Challenge experiments in rhesus monkeys

Complete ORF2 gene and the NE region cloned in pVAX1 and corresponding proteins expressed in baculovirus and prokaryotic systems respectively were evaluated as vaccine candidates in rhesus monkeys. The approaches tried included DNA, DNA-prime-protein-boost (DPPB) and encapsulation of DNA and the corresponding protein in liposome (liposome).As the challenge inoculum was not pre-titrated in rhesus monkeys, number of HEV RNA copies was determined employing real time PCR and was estimated to be 10^7 GE/ml of the 10% stool suspension. Considering real time PCR to be ~10-fold more sensitive than infectivity in monkeys, the challenge dose was decided to be 10^{-3}. Figure 5 depicts dynamics of HEV infection in control, unimmunized monkeys inoculated with different doses of the virus. Both monkeys inoculated with 10^{-3} (MM# 215 and 216) and 10^{-4} (MM# 217 and 218) dilutions each and the only monkey infected with 10^{-5} dilution (MM# 219) showed evidence of HEV infection as indicated by seroconversion to anti-HEV antibodies and excretion of the virus in faeces. Rise in serum ALT (> two-times the pre-virus-inoculation levels) was recorded for MM# 215, 216, 218 and 219. The monkey (MM# 220) inoculated with 10^{-6} dilution remained IgG-anti-HEV negative. Thus the infectivity titre of PM2000 was estimated to be 10^6 monkey 50% infectious doses (MID_{50}) per gram of faeces and the challenge inoculum contained $100MID_{50}$ HEV.

Anti-HEV response in immunized monkeys

Table 5 provides details of the immunogens under evaluation . All the pre-immune sera taken prior to the first immunization were negative for IgG-anti-HEV. None of the monkeys receiving three doses of ORF2 DNA (MM# 201, 202) or NE DNA (MM# 205, 206) or two doses of ORF2 DNA and one dose rORF2p (MM# 203, 204) showed the presence of anti-HEV antibodies. The monkeys immunized employing NE DNA-prime-protein-boost approach (Group1, MM# 207, 208) did not develop anti-HEV antibodies after two DNA

doses. Serocoversion was noted four weeks after the protein boost (titres 1:1600 and 800). Of the 4 monkeys receiving ORF2-liposome (Group 2, MM# 209-212), one seroconverted 3 weeks after the first dose (anti-HEV titre 1:100), all four being anti-HEV positive one week after the second dose (anti-HEV titres: 1:100-800). Both the monkeys receiving NE-liposome (Group 3, MM# 213, 214) seroconverted 3 weeks after the first dose, anti-HEV titres being 1:800 and 1:1100 respectively. One week after the second dose, the anti-HEV titres rose to 1:6400 and 1:800. Thus, for each group, the pattern of serum antibody was similar in all the monkeys, although antibody level was different. As compared to ORF2, anti-HEV titres produced by NE were higher, though statistically insignificant (p=0.053) (Figure 4).

Dynamics of HEV infection in control monkeys

Both the monkeys infected with 100 MID_{50} virus used for challenge of the immunized monkeys exhibited moderate rise in serum ALT levels, maximum values being 77 and 42 IU/litre on 38 and 45 days post-inoculation respectively (figure 3). Virus excretion as measured by Real Time PCR was evident for 6 weeks, the maximum viral load being $1.5X10^7$ and $6.0X10^6$ GE /g stool. Seroconversion followed by high titres of anti-HEV antibodies were recorded.

Table 5: Details of immunogens and dose of HEV

Group, Mky No	Dose 1	Dose 2	Dose 3	Challenge / dose
1, MM# 201, 202	ORF2 DNA 20μg	ORF2 DNA 20μg	ORF2 DNA 20μg	NO[a] / NIL
2, MM# 203, 204	ORF2 DNA 20μg	ORF2 DNA 20μg	rORF2p 20μg	NO / NA
3, MM# 205, 206	NE DNA 20μg	NE DNA 20μg	NE DNA 20μg	NO[a] / NA
4, MM# 207, 208	NE DNA 20μg	NE DNA 20μg	rNEp 20μg	YES / 10^{-3}
5. MM# 209, 210 211, 212	NIL	ORF2 DNA 20μg + rORF2p 20μg + liposome	ORF2 DNA 20μg + rORF2p 20μg + liposome	YES / 10^{-3}
6. MM# 213, 214	NIL	NE DNA 20μg + rNEp 20μg + liposome	NE DNA 20μg + rNEp 20μg + liposome	YES / 10^{-3}
7. MM# 215, 216	NIL	PBS	PBS	10^{-3}
8. MM# 271, 218	NIL	NIL	NIL	10^{-4}
9. MM# 219	NIL	NIL	NIL	10^{-5}
10. MM# 220	NIL	NIL	NIL	10^{-6}

Monkeys seroconverted only after 4th DNA dose

Assessment of HEV infection in challenged monkeys

Monkeys not developing anti-HEV antibodies following immunization were not challenged. These included macaques immunized with ORF2 DNA, NE DNA and ORF2 DNA-prime-protein-boost (MM# 201-206). Thus, NE DNA-prime-protein-boost and NE and ORF2-liposomes were evaluated as vaccine candidates. Irrespective of type of immunogen, none of the challenged monkeys exhibited raised ALT levels and were protected from hepatitis (Table 6). Complete protection from infection was offered by NE-liposome, both animals not excreting the virus (MM# 213-214). Similarly, one of the monkeys immunized with NE-DNA-prime-protein-boost (MM# 207) also did not show any evidence of virus replication, HEV RNA being absent in all the faecal samples screened. The other animal (MM# 208) showed reduced excretion for a shorter time. ORF-2 liposome was least effective, all the 4 monkeys (MM# 209, 210, 211, 212) excreting the virus for extended period of time. As compared to the control monkeys, overall viral load in faeces was significantly less in monkeys immunized with NE-DNA-prime-protein-boost and NE-liposome ($p<0.001$ for both); no difference was noted in ORF2-liposome immunized monkeys ($p>0.4$). Among NE-immunized groups the difference was non-significant ($p>0.4$). Comparison of IgG-anti-HEV titres in the challenged monkeys showed that except for MM# 209 and 211 immunized with ORF2-liposome, all other monkeys exhibited either same or declining antibody titres. Anti-HEV titres in MM# 209 and 211 increased to levels similar to control monkeys strongly suggesting replication of the virus leading to the boosting effect.

Anti-N-ORF2 antibodies as indicator of HEV infection in immunized monkeys

To identify HEV infection in immunized and challenged monkeys, an ELISA using ORF2 protein containing N-terminal 111 amino acids (N-ORF2) was standardized. N-terminal segment of ORF2 gene (333 nt, representing N-terminal 111 aa) was cloned in vector pET15b in frame with 5'-His tag, protein was induced by adding 1mM IPTG and purified using ProBond resin (Invirogen). This protein was used for coating the ELISA wells. Control monkeys infected naturally with HEV exhibited seroconversion to ORF2 (112-607/660) and N-ORF2 proteins on the same day (Figure 3). None of the monkeys immunized with NE in different formats developed anti-N-ORF2 antibodies either after immunization or challenge suggesting absence of natural infection in these animals. It is interesting to note that all the 4 monkeys immunized with the complete ORF2 DNA and 55kDa protein encapsulated in liposomes did not develop anti-N-ORF2 antibodies. Immune response was mainly targeted against the 112+ protein component. However, MM# 209 and 211 showing > 10-fold rise in anti-ORF2

antibodies after challenge showed the presence of anti-N-ORF2 antibodies . Rise in anti-HEV titres and the detection of anti-N-ORF2 antibodies were simultaneous. Anti-N-ORF2 antibodies were not detected in the other two monkeys immunized with ORF2-liposome (MM# 210, 212) and all the 4 monkeys immunized with NE (MM# 207, 208, 213, 214). These results clearly demonstrated that two of the four monkeys immunized with ORF2-liposome developed HEV infection following challenge whereas all the four monkeys immunized with NE were protected. The results document NE to be a promising vaccine candidate, especially as a liposome preparation entrapping DNA and protein.

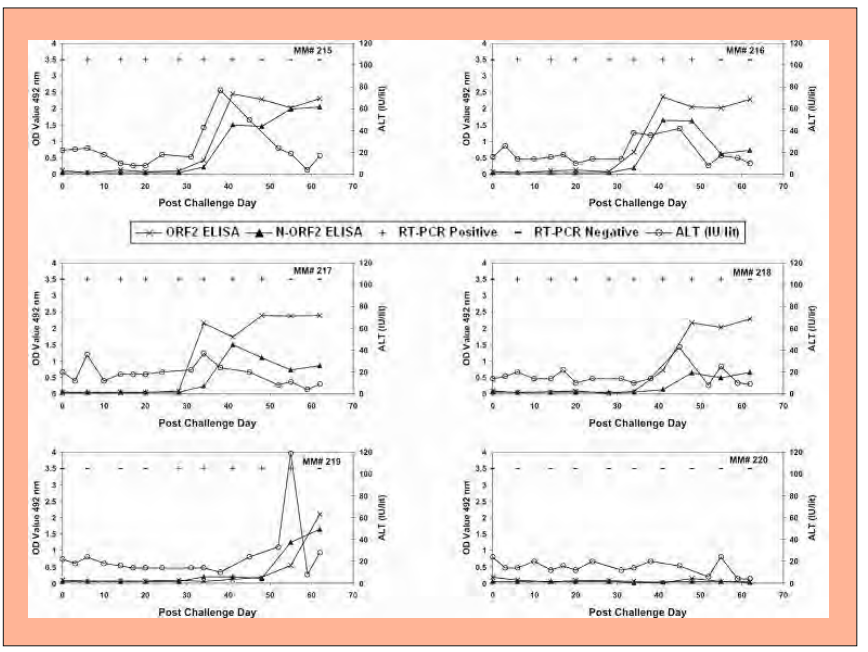

Figure 3: Anti-HEV titres in monkeys immunized with NE-DNA-prime-protein-boost (group 1: 2 monkeys), ORF2-liposomes (group 2: 4 monkeys) and NE-liposomes (group 3: 2 monkeys). Groups 2 and 3 were immunized with 2 doses. Antibody titres were determined 4 weeks after each dose.

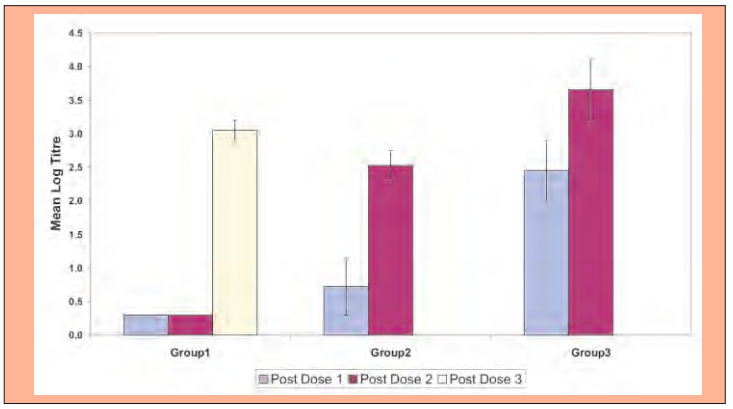

Figure 4: Anti-HEV titres in monkeys immunized with NE-DNA-prime-protein-boost (group 1: 2 monkeys), ORF2-liposomes (group 2: 4 monkeys) and NE-liposomes (group 3: 2 monkeys). Groups 2 and 3 were immunized with 2 doses. Antibody titres were determined 4 weeks after each dose.

Table6: Summary of challenge experiment

Monkey (Group)	Vaccine type	Reciprocal anti-HEV titre at challenge	Peak/ pre challenge ratio of ALT values (weeks elevated)	HEV genomes in faeces Log_{10} peak titre (Duration in weeks)
207(A)	NE DNA prime-protein boost	1600	1.0 (0)	Not detected
208(A)		800	1.7(0)	$2.2X10^4$ (3)
209(B)	ORF2 DNA +ORF2 protein + liposome	200	1.1(0)	$2.9X10^6$ (6)
210(B)		1600	1.09(0)	$1.0X10^4$ (3)
211(B)		200	1.09 (0)	$2.6X10^6$ (5)
212(B)		200	1.35 (0)	$1.0X10^4$ (6)
213(C)	NE DNA + NE protein + liposome	12800	1.06 (0)	Not detected
214(C)		1600	1.06 (0)	Not detected
215(D)	Placebo	<10	3.6 (2)	$1.5X10^7$ (6)
216(D)		<10	2.1 (1)	$6.0X10^6$ (6)

Immunogenicity of genotype-specific formulations of NE

In order to understand the role of protein and DNA components of the vaccine in the liposome preparation, a preliminary experiment was performed employing following combinations: (1) Type 1 NE DNA + Type 4 rNEp (T1NED + T4NEP), (2) Type 4 NE DNA + Type 1 rNEp (T4NED + T1NEP) (3) Type 4 NE DNA + Type 4 rNEp (T4NED + T4NEP) and regularly used Type 1 NE DNA + Type 1 rNEp (T1NED + T1NEP). 6-8 weeks old female Swiss albino mice (n=10) were immunized with 2 doses (1+1µg/dose) of pVAX1+NE plasmid DNA construct and rNEp formulated in liposomes as given above. The experiment is under progress.

Development of Combined DNA Vaccine for Hepatitis B and E viruses

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As part of the final goal of developing a combined vaccine for hepatitis A, B and E, an attempt was made to evaluate combined B and E DNA vaccine. As reported earlier, “S” gene of Hepatitis B virus and complete ORF2 or truncated “NE” region of HEV were separately cloned in a mammalian expression vector pVAX1. This year, “S” and “NE” genes were cloned together in a single pVAX1 vector with the inclusion of IRES region or dual CMV promoter.

On the background of the promising results observed in mice immunized with a liposome preparation containing ORF2 DNA and protein, the same approach was tried for combined B+E vaccine candidate. Eight groups of 6-8 weeks old Swiss Albino mice were immunized subcutaneously with two doses of different

liposome preparations 4 weeks apart: (1) NE-liposome, (2) S-liposome, (3) NE + S-liposome and (4) pVax1-liposome. These preparations were given with or without $Al(OH)_3$. The results are shown in table 7. Figures 5 and 6 depict percent anti-HBs and anti-HEV positivity respectively at different time points. In mice immunized with both NE and S liposome together, early sero-conversion was observed for both antigens as compared to the respective single antigen. $Al(OH)_3$ was found to enhance humoral immune response with early sero-conversion and high antibody titers.

Table 7. Percent Seroconversion in mice immunized with different liposome formulations

Liposome formulations		% sero-conversion one week post dose 3
NE	a) with Al.hydroxide	100%
	b) without Al.hydroxide	100%
S	a) with Al.hydroxide	50%
	b) without Al.hydroxide	100%
NE+ S	a) with Al.hydroxide	90%(for anti-HBs) 100%(for anti-HEV)
	b) without Al.hydroxide	80%(for anti-HBs) 100%(for anti-HEV)
pVAX1	a) with Al.hydroxide	0%
	b)without Al.hydroxide	0%

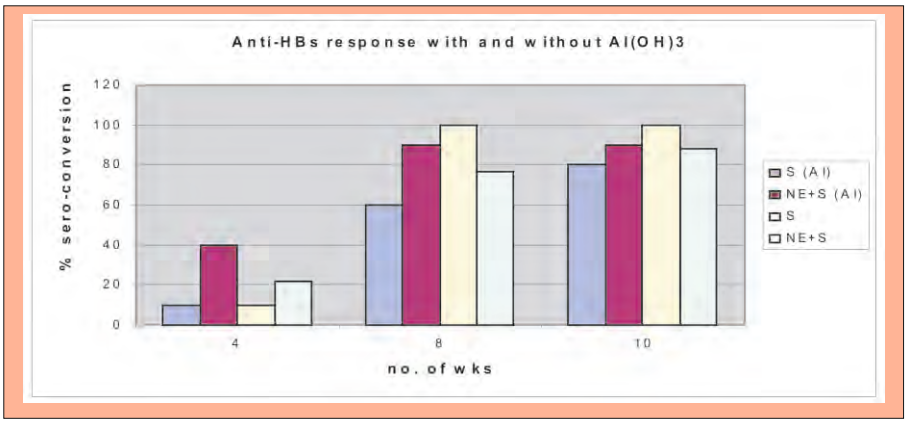


Fig 5: Percent sero-conversion in mice immunized with S-liposome alone and combined with NE-liposome

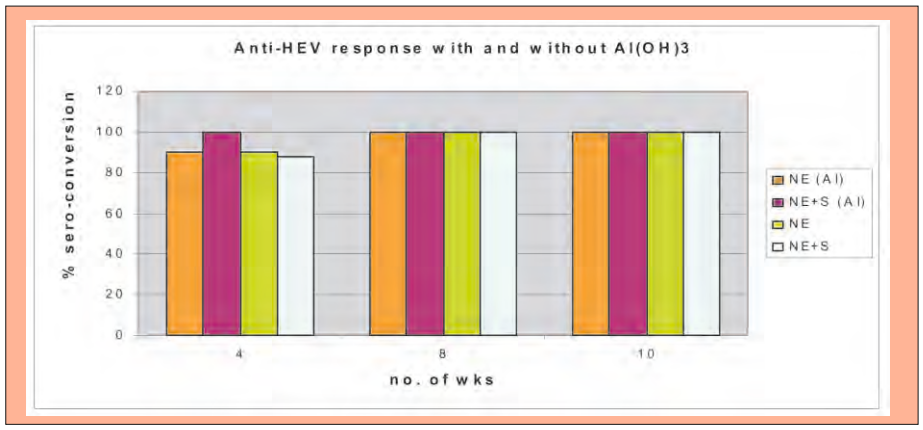


Figure 6: Percent sero-conversion in mice immunized with NE-liposome alone and combined with S-liposome

Generation of infectious cDNA clones for swine and human HEV

KS Lole, VA Arankalle

The aim of this project was to develop a chimeric full-genome constructs of type1 and type4 HEV to assess involvement of different viral genes in the pathogenesis and replication of the virus. Full genome cloning of genotype 1 HEV was completed in the current year. For that initially a different strategy was being followed in which HEV genome was amplified in nested PCR reactions as five overlapping segments, TA-cloned separately, and put together using restriction enzyme sites in the overlapping regions. We could construct a full-genome long clone with this but it had multiple single nucleotide deletions. To overcome this problem PCR amplified fragments were put together by stepwise stitching PCRs to obtain full-length product, which was then TA-cloned. Clones were screened initially by restriction enzymes. Five clones were with forward orientation and four clones with reverse orientation. One reverse orientation clone was processed for full-genome sequencing and it showed one deletion at 6024 position (ORF2 region). Second reverse orientation clone was checked for the same deletion and after confirming it to be absent the complete clone was sequenced. There were total 50 nucleotide changes as compared to the original full-genome sequence of the same virus. None of the changes caused premature termination of the ORFs. Plasmid was linearised at 3' end with BamH1 and in vitro run off transcription was done. RNA transfection experiments were done in RD and HepG2 cells with 5, 10, 15 and 20 µg RNA using different concentrations of DMRIE-C reagent. Virus replication was monitored using immunofluorescence staining of the cells and the cell lysates were processed for immunoprecipitation followed by SDS-PAGE

analysis. None of the transfection experiments indicated HEV replication. Genotype 1 HEV full-genome is being cloned once again to reduce the total number of single point mutations.

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

The ORF1 of HEV codes for the viral nonstructural polyprotein with a molecular mass of ~186 kDa. Based on protein sequence homology, the ORF1 polyprotein is proposed to contain four putative domains indicative of methyltransferase (MeT), papain-like cysteine protease (PCP), RNA Helicase (Hel), and RNA dependent RNA polymerase (RdRp). Of these, the MeT and RdRp enzymatic activities have been demonstrated while activities of the Hel and PCP have so far not been elucidated. To study steps involved in the processing of the ORF1 polyprotein, protein domains were predicted using pfam and SMART prediction servers. Complete ORF1 region (5082nt) and individual predicted domains of MeT, PCP, Hel and RdRp were PCR amplified using type 1 HEV full-genome clone and cloned in pET15b vector for E.coli cell expression. Cloning and expression of complete ORF1 polyprotein and helicase domain protein were completed. Single step purification of Helicase domain protein (25kDa) was done using Ni-resin column and optimum purity of the protein was obtained at 500mM immidazole concentration in the elution buffer. On 15% SDS-PAGE analysis this purified protein showed a single band of expected size (~25kD). Purification ORF1 protein (180kDa) is ongoing. Both proteins will be assayed for helicase activity.

Molecular evaluation of water samples and water treatment protocols with special reference to Hepatitis A & E viruses

Vikram Verma, V A Arankalle

Elution of viruses adsorbed to membrane filter

Significant amount of virus was being adsorbed to membrane filter during amicon concentration step and this was main cause of low efficiency and low reproducibility of our two-step concentration protocol. Various solutions were tested to elute the adsorbed viruses. Urea-Glycine solution was found to be most effective eluent. With the use of urea-glycine elution, we could recover ~80 % of the spiked virus and the results were reproducible.

Collection and screening of drinking water samples

After all the required techniques for the concentration and detection of viruses were standardized, we initiated collection of drinking water samples from three water treatment plants from Pune and some points of common public use. Fifteen forty samples were collected weekly, 6 from water treatment plant (affluent and effluent) and 9 from points of common public use. 40 liter of water sample was collected and concentrated up to 4 ml using ultrafiltration technique reported earlier. Concentrated water samples were subjected to nested multiplex PCR for detection of HAV, HEV, Enteroviruses and Rotavirus. Out of 107 drinking water samples screened so far, one sample was positive for Hepatitis A virus and 2 samples were positive for enteroviruses.

Identification and characterization of animal Hepatitis E virus(es)

V.A.Arankalle

Transmission of hepatitis E following consumption of raw meat and detection of HEV RNA in the pig liver samples from grocery shops has been reported from Japan. To understand the situaton in India, a study was initiated. Pig Livers were purchased from local market. So far, none of the 28 samples tested for HEV RNA was positive.

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

Arankalle VA

During this year, 226 sporadic hepatitis A and 49 sporadic hepatitis E cases diagnosed at the National Institute of Virology on the basis of the presence of IgM-anti-HAV antibodies were investigated. Of the 226 IgM-anti-HAV positives, 149 were tested for HAV RNA and 136 were scored positive. HEV RNA positivity was recorded in 28 of the 49 HEV-IgM positives screened. All PCR positives were sequenced. Based on phylogenetic analysis, all HAV samples belonged to genotype IIIA while the HEV samples were classified as genotype 1.

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

Nischay Mishra, Arankalle VA

The pathogenesis of fulminant hepatitis seen as a complication of HEV infection remains incompletely understood. The present study continues with the previous work to characterize at a cellular and molecular levels the mechanisms that HEV can contribute to severe liver failure.This year, two fulminant hepatitis E cases were investigated. Nearly complete genomes were amplified in overlapping fragments and sequenced. For cytokine polymorphisms analysis, samples were collected from 40 healthy individuals and 22 acute hepatitis E cases. The TNF-gene polymorphism is being studied employing ABI PRISM SNaPshottm Multiplex protocol.

Study of humoral and cell-mediated immune responses in Hepatitis B vaccinees

Anuradha Tripathy, Arankalle VA

Health care workers (HCW) at risk of exposure to the HBV need to be vaccinated. Approximately, 4-10% of healthy vaccinated individuals fail to produce protective levels of anti- HBV antibodies after standard vaccination. To assess the role of host factors towards non-responsiveness to vaccine, we studied HBsAg specific cytokines, CD4 T cell response and frequencies of HLA Class II alleles in 50 HCW, vaccinated with HB vaccine. Assessment of cytokines levels was carried out from stimulated PBMCs by ELISA, HBsAg specific memory T cell response was studied using lymphocyte proliferation assay and the frequencies of HLA Class II alleles was determined by PCR SSP method. The results showed significant levels of IFN-γ and IL-12 and HBsAg specific CD4 T cell response in vaccinees despite absence of specific antibodies. Frequencies of alleles DRB1* 15 and DQB1*05 significantly increased in vaccine responders compared to controls.

Multi-centric drug trial for hepatitis C

V.A.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”. The NIV is responsible for the Virological component. This year, a total of 206 samples of 142 patients were tested for HCV RNA in nested RT-PCR. Core-gene sequence-based genotyping was carried for 59 samples. HCV RNA Quantitation was carried out for 236 samples using Amplicor HCV Monitor test.

Services provided

1. Testing for Drug Controller of India: A total of 403 blood products submitted by the drug controller of India were tested for HBsAg and HCV RNA and reports submitted.
2. Chronic Hepatitis B and C patients: A total of 41 and 50 patients were tested for the presence of HCV RNA and HBV DNA respectively in PCR. & HBV Quantitation was done for 31 patients
3. Sporadic acute viral Hepatitis Patients: A total of 413 and 299 and 144 serum samples were tested for the detection of anti-HAV-IgM, anti-HEV-IgM and HBsAg respectively.
4. Epidemics of viral Hepatitis: 296 sera representing 6 outbreaks of viral hepatitis were tested.

In vitro studies on growth and characterization of Indian isolates of Hepatitis A virus (HAV)

SD Chitambar, RS Fadnis

Two isolates of HAV were adapted to Vero E6 cell line and serially passaged upto passage 20.

Objective

To characterize the HAV isolates.

Work done

Growth characterizatics of one of the isolates (NP) have been documented in earlier annual report. In continuation, the growth of second isolate (PV) was monitored by IFA at virus passage 18. The HAV antigen was detected in 10 day old cell cultures. The S/N ratios in antigen capture ELISA ranged from 1.9 to 20.7 up to virus passage 20. The infectious titer of the isolate was > 1:625 in normal Vero E6 cells.

Genomic characterization of adapted isolates NP and PV was carried out at passage 18 in selected regions of the HAV genome i.e 5' NCR, 2B & 2C, responsible for in vitro adaptation. The comparison of the sequences in corresponding regions with cell culture attenuated HM-175 strain revealed: (1) Four substitutions for NP strain while 3 substitutions for PV strain in 5'NC region. (2) Eight nucleotide substitutions for NP strain and 4 substitutions for PV strain in 2B region. The deduced amino acid sequences showed 3 substitutions in each of the strains. (3) Nine nucleotide substitutions for both the strains in 2C region of the genome. The deduced amino acid sequence showed 6 substitutions in this region for both the strains.

Concentration of tissue culture adapted HAV isolates, NP and PV was carried out using polyethylene glycol precipitation method. The precipitated fractions were dialyzed and used for characterization of viral proteins. Western blot analysis using convalescent phase serum from hepatitis A patient detected four viral proteins with molecular weight 55KD, 40KD, 33KD and 29KD. The pH of these proteins was in the range of 4.55 and 5.85 as revealed by 2D-PAGE analysis.

Two isolates were found useful as an antigen in anti-HAV IgM capture ELISA carried out using serum samples known to contain anti-HAV IgM antibodies. Blocking ELISA performed using human and animal sera containing anti-HAV antibodies showed neutralization of two isolates.

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.

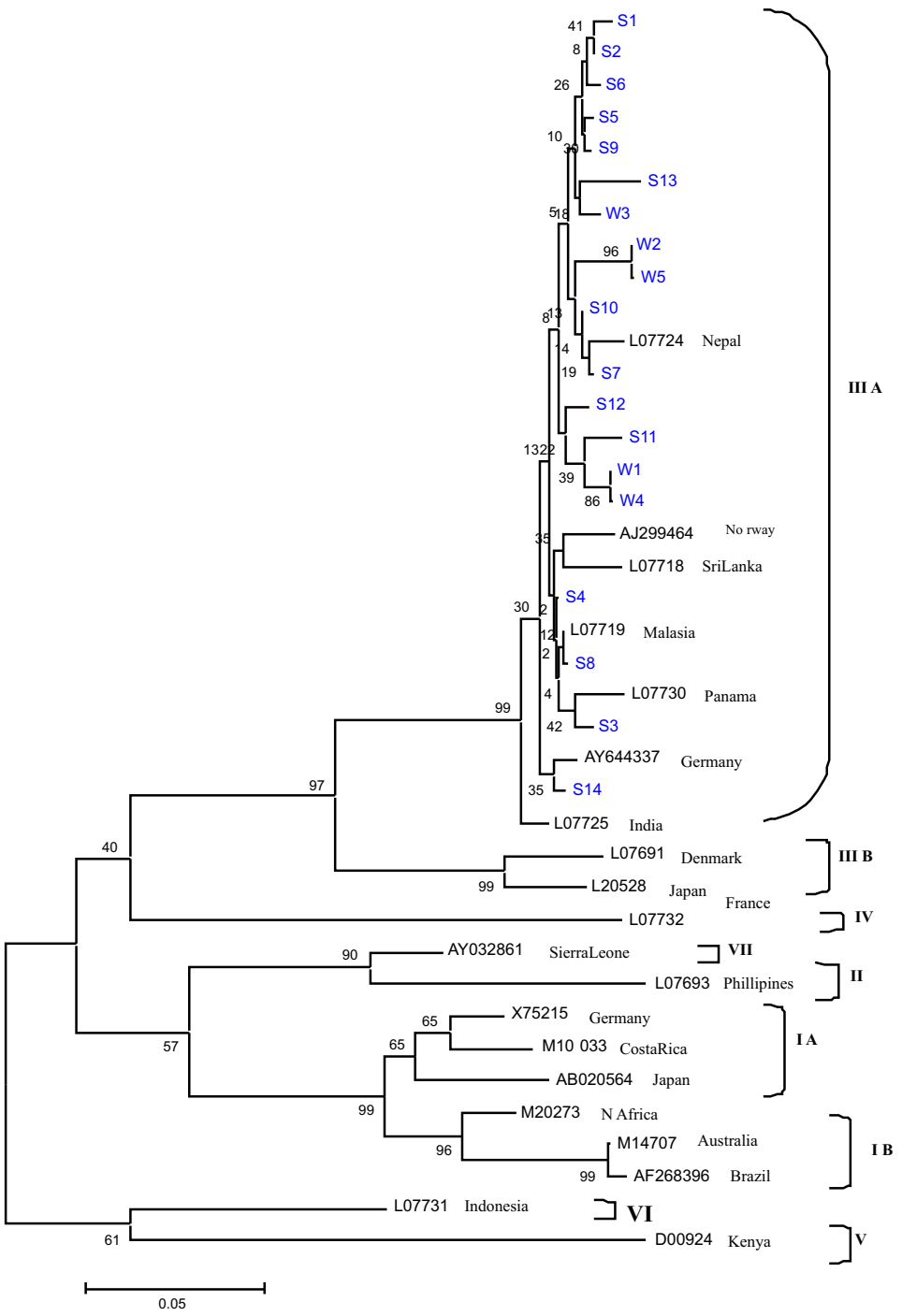
Objective

To characterize wild type hepatitis A virus strains recovered from different parts of India using VP1/2A junction region and partial RNA polymerase region and to sequence entire genome of representative HAV strains.

Work done

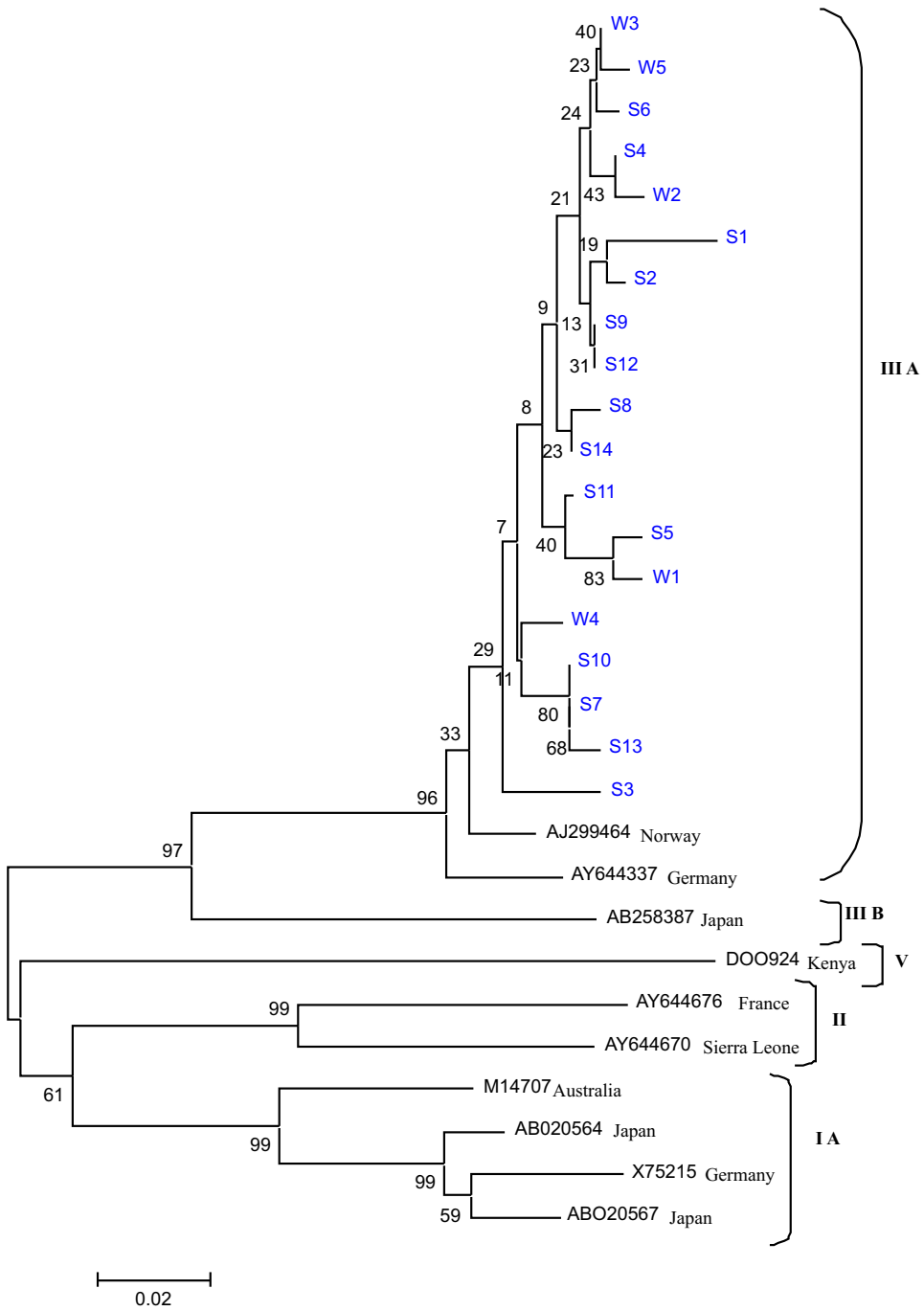
To determine the HAV genotypes prevailing in India stool and serum samples were collected during 2004-06 from western (n=5) and southern (n=14) parts of the country. Genomic analysis using VP1/2A junction region indicated genotype IIIA as prevailing genotype (Fig.1). Analysis of partial RNA polymerase region showed clustering of Indian strains with HAV strains from Norway and Germany (Fig. 2). Full-length genomes of genotype IIIA of HAV from Indian subcontinent were sequenced for the first time. Out of three strains sequenced two were recovered from patients with self limiting disease (CP-IND and PN-IND) whereas GBS-IND was the strain associated with GBS related neuropathy. Nor21 strain of sub genotype IIIA was used as the reference strain. Percent nucleotide identity (PNI) was 97.05 for CP-IND strain, 96.95 for PN-IND strain and 96.27 for GBS-IND strain. Within Indian strains PNI was in the range of 96.50-97.78. Comparison of deduced amino acid sequences of complete coding region with that of Nor21 strain showed percent amino acid differences (PAAD) of 0.1 with CP-IND and PN-IND and of 0.5 with GBS-IND. PAAD was 0.1 when Indian strains CP-IND and PN-IND were compared with each other. GBS-IND strain showed PAAD of 0.4 & 0.5 in comparison to CP-IND and PN-IND strains respectively.

Figure 1 : Genetic relatedness of wild type HAV isolates from Southern and Western India with other HAV strains in VP1/2A junction region(30243191)



S = South India W = West India

Figure 2 : Analysis of wild type HAV isolates from Southern and Western India in RNA polymerase region(6457-6572)



Additional Studies

Evaluation of animal anti HAV antibodies for neutralization of HAV

SD Chitambar, MS Joshi

Earlier studies carried out on animal sera indicated anti HAV prevalence at significant level in cattle, goats, dogs, pigs and rodents. Tissue culture adapted HAV at different dilutions was incubated respectively with each of serum samples from sero negative and positive buffalo and goat and inoculated in normal BGMK cell line. Significant inhibition in replication of HAV was noted after treatment with anti-HAV positive sera indicating presence of anti HAV neutralizing antibodies in both buffalo and goat.

Cultivation of HAV in embryonated egg system

MS Joshi, Shilpa Bhalla, LR Yeolekar, SD Chitambar

Hepatitis A virus is usually difficult to adapt and grow in vitro. Some wild type strains have not been adapted to cell cultures despite intensive efforts. Successful isolation from clinical specimens has been reported in monkey kidney derived cell lines. However, very slow growth of virus has been noted. Attempts to grow the virus in embryonated eggs were rarely reported. Objective of the study was to explore the possibility of cultivation of HAV in SPF embryonated eggs. The SPF eggs were procured from PDRC, Loni, Pune. The virus was passaged serially for 5 times. Supernatant of 10% fecal specimen from hepatitis A patient and tissue culture adapted strain were inoculated separately through allantoic,amniotic+ allantoic routes. No growth of HAV was monitored by antigen capture ELISA.

Publications

01. Arankalle VA, Shrivastava S ,Cherian S, Gunjikar RS, Walimbe AM,Jadhav SM, Sudeep AB, Mishra AC.Genetic divergence of Chikungunya Viruses in India (1963-2006)with special reference to the 2005-2006 explosive epidemic. *J Gen Virol* 2007, 88: 1967-76.

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05 Capoulade-Metay C, Aoyuba A, Kfutah A, Lole K et al. A natural CCL5/RANTES variant antagonist for CCr1 and CCR3. *Immunogenetics* 2006; 58: 533-41.

06 Chitamber S, Joshi M, Lole K, Walimbe AM, Vaidya S. Cocirculation of and coinfections with hepatitis A virus subgenotypes IIIA and B in patients from pune, Western India. *Hepatol Res* 2007, 37 : 85-93.

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07. Lole KS, Arankalle VA. Quantitation of hepatitis B Virus DNA by real-time PCR using internal amplification control and dual TaqMan MGB probes. *J virol Method* (2006) 135 : 83-90

09 Mavale MS, Fulmali PV, GeevargheseG, Arankalle VA,Ghodke YS,Kanojia.PC, Mishra AC Veneral transmission of Chandipura Virus by Phlebotomus papatasi (scopoli). *Am J Trop Med Hyg.* 2006, 75 (6)1151-52

10. Yergolkar PN, Tandale BV, Arankalle VA et al. Chikungunya outbreaks caused by African genotype, India. *Emerg Inf Dis* 2006: 12; 1580-1583

Participation in meetings, workshops

Arankalle V A

Indo-German meeting 10-12 June 2006 at Kolkata

12th International symposium on viral hepatitis and liver disease 1-5 July 2006 at Paris, France.

Project Review Committee meeting, 31st July 2006 at ICMR, Delhi

Invited speaker during Indo US symposium entitled," Emerging and re-emerging infectious diseases and disease surveillance". Topic: Hepatitis E surveillance at 2nd medical development congress: Genomics and proteomics in health and disease 8th Sept 2006 at Delhi

DBT-CDC meeting on avian Influenza 9th Sept 2006 at Delhi

Meeting of the multi-centric drug trial project for chronic hepatitis C 27th Sept 2006 at ICMR

Invited speaker on "Enteric viruses" during a symposium, " Water borne infections: Causes and Control" at the annual meeting of the Indian Association of Medical Microbiologists 26-28 Oct 2006 at Nagpur:

Invited speaker on "Hepatitis B" during the Indo-US Symposium 11-12 Nov 2006 at Delhi

Invited speaker during Asia Pacific congress of Medical virology. Topics: HEV diagnosis and Chikungunya in India13-15 Nov 2006

Invited speaker, "HBV mutants and genotypes in India" 11th Feb 2007 at CMC, Vellore

Meeting of the co-ordinators of the project, "A multicentric randomized controlled clinical trial of Adefovir, Lamivudine, and Glycyrrhizin in HBV related decompensated cirrhosis" 6th March 2007 at ICMR, Delhi

Invited speaker during Sir Dorabji Tata Symposium on Arthropod borne viral infections, , "Molecular Biology of Chikungunya" 10th March 2007 at I.I.Sc., Bangalore

Japanese Encephalitis


LABMATE 2007

Japanese Encephalitis Virus Research Group

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Phylogenetic analysis of JE virus isolates in India

Vijay P. Bondre, PN Yergolkar, G. N. Sapkal, P. Fulmali, R. S. Jadi and M. M. Gore.

Evolution of JE virus has ocurred over a suggested scale of over 130 years and based on a 12% divergence in a stretch of 140 or 198 nt fragment spanning the C-preM region, JE viruses have been classified in to four genotypes. Viruses isolated from most of the Asian countries are grouped in different clusters of genotype III. Classification of JE virus strains using a short fragment (<200 nt from C/preM), might generate incorrect tree topologies during phylogenetic analysis. As a RNA virus, JE virus is prone to micro evolutionary events occurring at genomic level leading to strain variations. Co-circulations of different genetic variants, and their introductions in newer geographic areas have been documented. Genetic and antigenic variations among JE virus strains isolated from different geographic areas of India have been also documented. The present study was initiated to determine the possible genetic relationship among JE viruses isolated during last 50 years in India.

Objectives

To determine the genotypes of JE virus strains isolated during last 50 years in India.

To determine the genetic relationship of Indian JE virus isolates

To determine the utility of partial C-preM and envelope region sequence in phylogenetic analysis of JE virus

Methodology

This study analyzed the genetic relationship among 37 JE virus strains isolated from different host species from different geographic regions of India during last 50 years (1956-2006). We determined the genetic relatedness of these viruses using a 366 nt sequence from C/preM region (spanning the 198 nt fragment originally used for genotyping) and compared with globally reported JE virus strains from GenBank. In addition, the genetic relationship among Indian (37 isolates) and globally reported JE virus strains was determined by using a 680 nt fragment from envelope gene. The JE virus from Japan, China and Sri Lanka available with NIV repository served as referral controls during this study.

Findings

The C-preM sequence analysis classified all the JE virus strains isolated in India along with Sri Lankan, Nepalese and Chinese referral strains classified in

genotype III. Phylogenetic analysis further divided the Indian isolates in two distinct genetic clusters with 6-7% genetic divergence. The cluster 1 was further divided in to different groups based on the year of isolation and geographic locations. Older strains (1956-78) isolated from southern geographic regions of India formed distinct group within cluster 1. The Sri Lankan and Japanese referral strains along with one Indian isolate (Karnataka, 1982) formed separate group within cluster 1. A human isolate of 1990 from Karnataka formed separate group within cluster 1. Twelve JE virus isolates from UP (5), MP (1), MH (2), WB (2) and AS (2) along with twelve isolates from KK (1980 -1991) and one isolate from Goa formed distinct cluster 2, within genotype III (Figure 1).The phylogenetic relationship with envelope region sequence analysis was similar to the C-prM region, except that the group V was further branched in to distinct branches. Except the strains from southern India, no correlation with time and geographic distribution is observed. Based on phylogram, all the Indian JEV strains grouped in to three major groups diverged by 3-6%. Group 1 - Older southern Indian group containing strains up to 1978; Group 2 - central, northern and western Indian group, & Group 3 - new southern Indian group including strain from Goa. Genetic distance between the southern and northern Indian JEV is in the range of 6.5 - 8%. The southern Indian JEV shows 97-100% sequence similarities among themselves. The northern Indian JEV shows 97-100% sequence similarities among themselves. 21% is the highest genomic divergence shown by an Indonesian JEV strains JKT6468 with most of the strains. Similarly the Australian FU strain also shows about 13-14% genomic divergence with other isolates (Fig. 2).

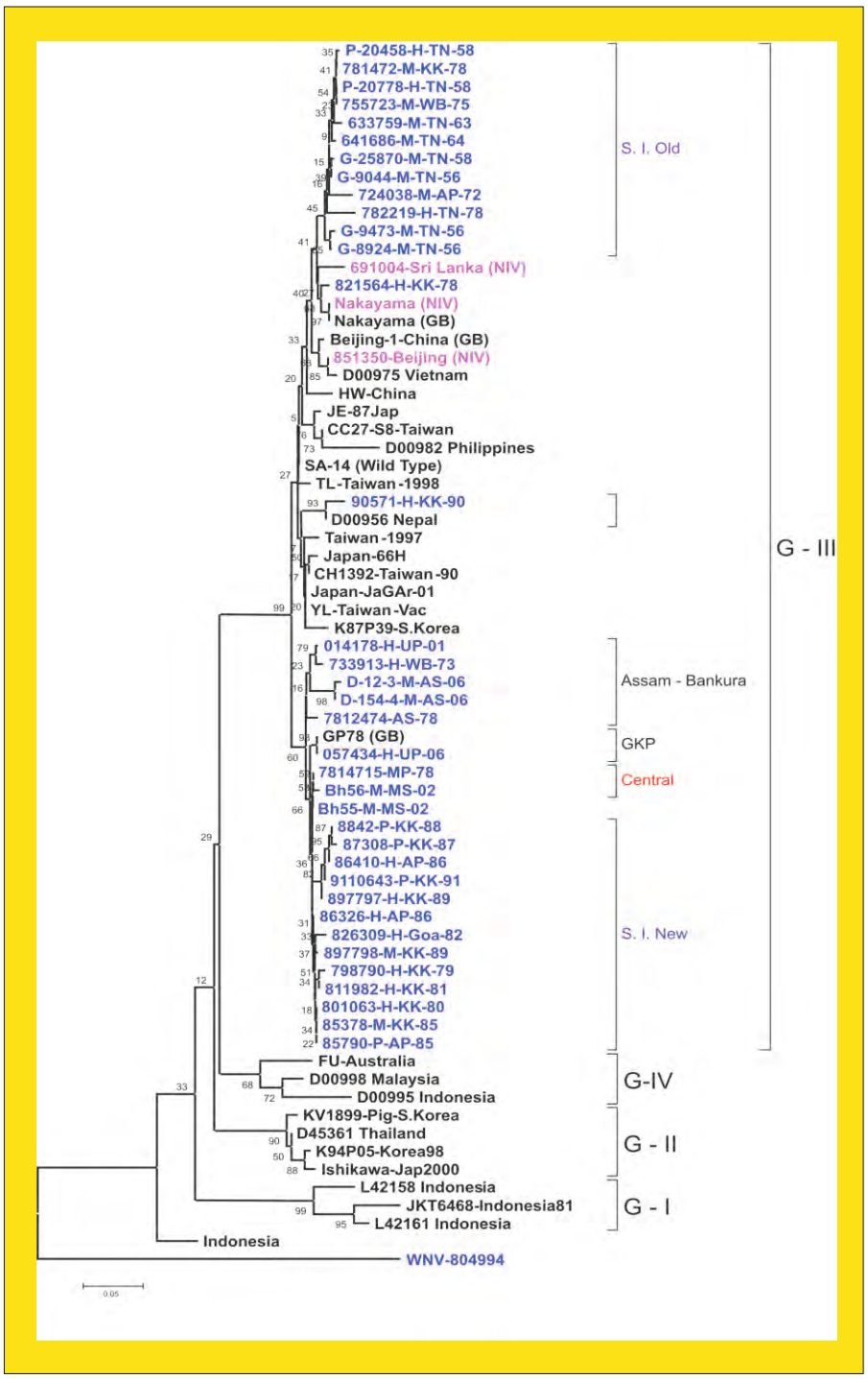


Fig.1: Genotypes III JEV strains isolated in India using 366 nt sequence from C-preM region.

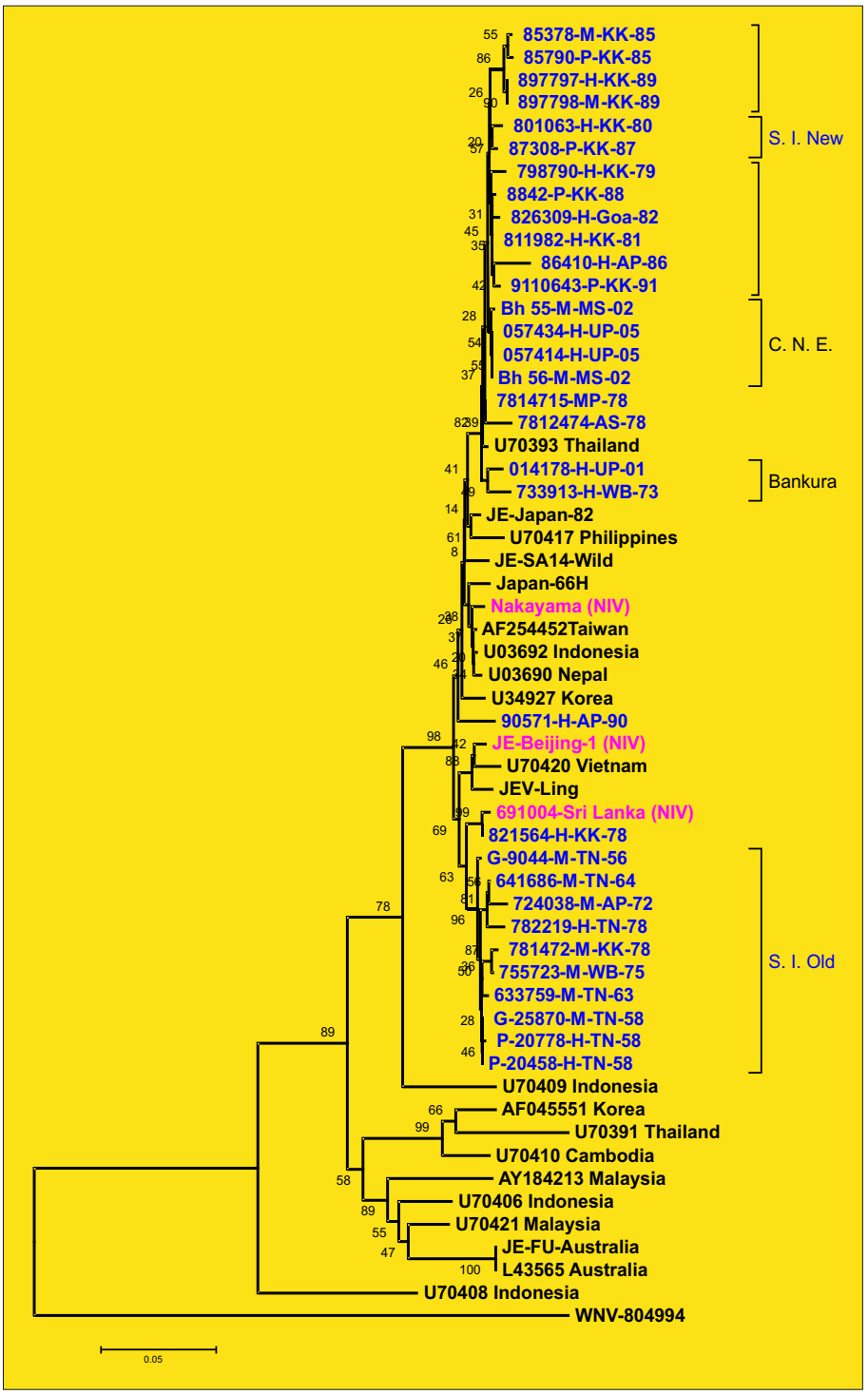


Figure 2. Phylogram of JEV strains isolated in India using 680 nt sequence from envelope region.

Complete genome sequencing of recently isolated JE virus strains from India

V. P. Bondre, V. Shankararaman, P. V. Fulmali, G. N. Sapkal and M. M. Gore.

Introduction

Till date, only two Indian strains (P-20778 of Vellore, 1958 & GP78 of Gorakhpur, 1978) have been completely sequenced and are available in GenBank. Our phylogenetic study based on partial genomic sequences revealed isolation of JE virus strain distinct from circulating strains in the country. To investigate any microevolutionary alterations occurring at genomic level that may result in selection of highly virulent strains, full genome characterization of newly isolated JE virus strains was undertaken. The study will highlight the extent of variation occurring in nature with respect to time.

Methodology

Full genome sequence of JE virus isolates 014178 from Lakhimpur (Human, UP, 2001), 057434 from Gorakhpur (Human, UP, 2005), 04940-6 from Bhandara (Mosquito, Maharashtra, 2002) and 90571 from Bellary (Human, Karnataka, 1990) was carried out. The overlapping sequences (from both the ends) obtained by RT-PCR amplification followed by sequencing were aligned to construct full genomic sequence.

Findings

The percent nucleotide identity analysis indicates close relationship of GP78 with 057434 (99.400.07) and 04940-6 (99.300.08). Comparatively 014178 (Bankura group) isolate was distinct GP-78 with the PNI of 95.970.19. The genetic distance of these strains with P20778 was in the range of 4-6%. Although the PNI of 90571 isolate with P20778 (southern India) and GP78 (northern India) ranges from 3-4%, phylogenetic analysis shows its close relationship with JE virus strains isolated from south Korea (Fig. 3).

Summary

Analysis of complete genome sequence of 4 JE virus isolates from various regions of India indicates that different strains are circulating in India. The older south Indian strains are closely related to Nakayama strain from Japan. Strains circulating in central and northern India are closely related to GP78. Strains forming the Bankura group are distinct from strains circulating in southern and northern India and are restricted to their respective geographic regions. Secondly the human isolate from Bellary (1990) seems to be an introduction of South Korean variant probably via Nepal.

Fig. 3. Full genome sequence based phylogenetic analysis of JE virus strain isolated in India.

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

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

Introduction

North east region is endemic for JEV. Very little information is available on the genotypes of JE virus circulating in that region. In addition a systematic study of vector population, infectivity and the serological response against JE virus is necessary. The project is a collaborative project between RMRC Dibrugarh and NIV.

Objectives

To study the prevalence, disease pattern, epidemiology and molecular characterization of JEV virus strains circulating in the NE parts of India.

Work Summary

Development of rapid tests for detection and characterization of JE virus

Real Time RT-PCR quantification of JE virus was standardized using lab based titrated JE virus strains including 733913, SA-14-14-2 vaccine strain using Geno Sen's JE virus Real Time PCR Standard Reagents supplied by Professional Biotech, Delhi. Reactions Performed on Rotor-Gene Machine and data analyzed by Rotor-Gene 6.0.33 Software. The specificity of the real time detection of JE virus was established by using West Nile virus as negative controls and titrated strains of JE virus as positive controls (Fig 4a)

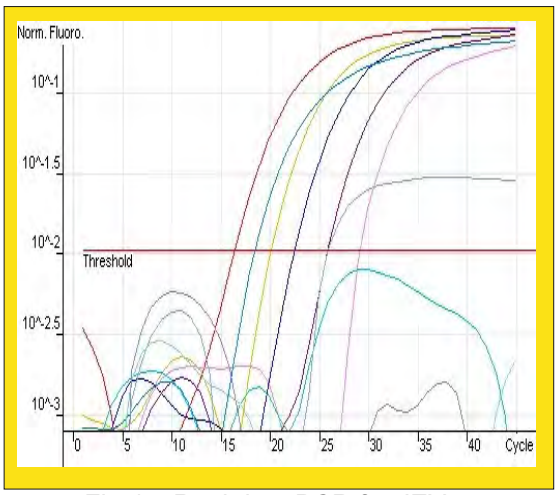


Fig.4a: Real-time PCR for JEV

Further the sensitivity to detect the JE virus strain SA-14-14-2 in blood specimens was performed by using various dilutions of the virus and co-related with the tissue culture based titration of same stock. The sensitivity of single tube real time RT-PCR assay to detect JE virus was established to 50 copies per reaction in 50µl volume (Fig 4b).

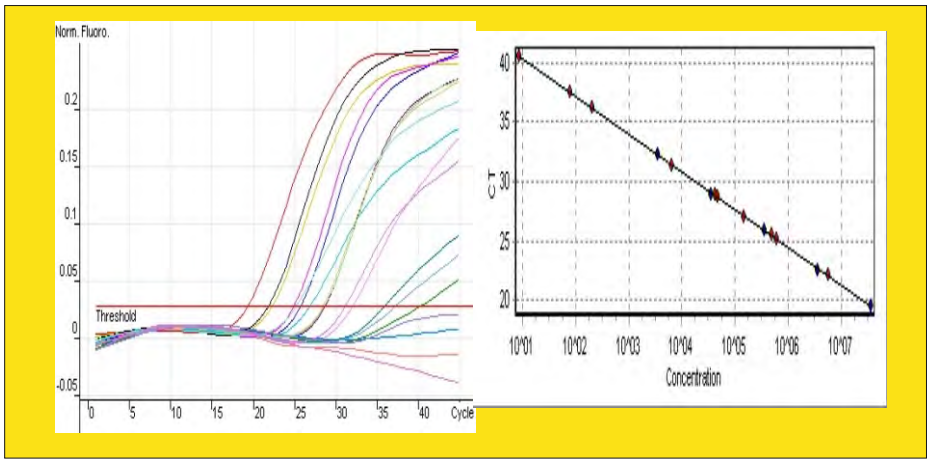
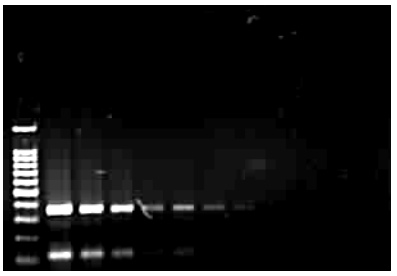


Fig.4b : Calibraton curves for JEV detection by real-time PCR

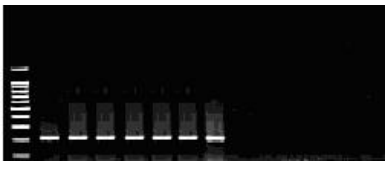
Detection of JE virus antigen and genome in field caught mosquitoes from North-East region of India

About 400 mosquito pools were received from North East. All pools were processed for antigen detection using MAb HS-2 for antigen capture and probed by biotinylated MAb HX2. Out of these 20 pools were found positive for JE virus. ELISA positive pools (5 at a time) were processed for isolation in infant mice and monitored for sickness. The harvested brain suspension (P1, 2Day PI , 5 day DPI) was also processed for isolation in BHK cell line. However isolation attempts were not successful up to 7Day PI. The harvested mouse brains and tissue culture inoculated virus was monitored for detection by RT-PCR. Three mouse brain passage 1 suspensions were RT-PCR amplified and sequenced. The phylogenetic analysis based on 400 nt sequence placed these isolated close to the GP78 virus. A comparative analysis of antigen capture ELISA, RT-PCR and Real time PCR was carried out on 60 mosquito pools comprising of 17 ELISA positive pools. Results indicated that two step RT PCR can be used for detection of JE virus from mosquito pools (Figures 5a and 5b and tables 1 and 2).



Lane		Lane	
1	100 bp ladder	7	10^{-5}
2	JE Neat 7.3 log	8	10^{-6}
3	10^{-1}	9	10^{-7}
4	10^{-2}	10	10^{-8}
5	10^{-3}	11	Nor M. Br
6	10^{-4}	12	Nor TC

Fig.5 (a): RT PCR for detection of JE virus (First PCR)



Lane		Lane	
1	100 bp ladder	7	10^{-7}
2	10^{-2}	8	10^{-8}
3	10^{-3}	9	Nor M. Br
4	10^{-4}	10	1st PCR Control
5	10^{-5}	11	Nor TC
6	10^{-6}		

(b) RT PCR for detection of JE virus (Nested PCR)

Table 1: Sensitivity and specificity of molecular tests

	Detection limit (pfu/ml)		
Virus Source	Single step RT-PCR	Two step RT-PCR	Real time RT-PCR
Mouse Brain	≥100	≤1	≥10
Cell Culture	≥103	≤1	≥10

Table 2: Comparison JE virus positivity from different clinical samples

Type of sample (No)	MAC-ELISA Positive (%)	Single step RT-PCR (%)	Two Step RT-PCR (%)	Real Time PCR (%)
Serum (33)	8(24.24)	1(3.03)	7(21.21)	7(21.21)
Blood clot (20)	5(25)	3(15)	5(25)	4(20)
CSF (32)	6(18.75)	nil	9(28.12)	8(25.0)

Determinants of peripheral pathogenecity of West Nile Virus

GN Sapkal, VP Bondre, MM Gore

Introduction

Pathogenicity of virus depends upon the cell tropism associated with envelope protein. Epitopes on E protein can be mapped, determining the sequence changes brought about by neutralization escape. It could help to understand mechanism of neutralization.

Rationale

Neutralization escape variant of different MAbs would be mutated in the site relevant to each MAb binding. Sequencing the E gene of mutant would be used to map the neutralization epitope of WN virus. Neutralization escape variants with altered peripheral pathogenicity and altered growth kinetics in Neuro 346 were analyzed in order to define determinants of pathogenicity in a given virus. In order to study the peripheral pathogenic determinants, neutralization escape variants of WN virus were selected and studied.

Objectives

To detect the nucleotide changes in the E protein of peripherally pathogenic WNV in comparison with non-pathogenic isolates of the virus.

Work done earlier

Peripherally pathogenic WN virus strain 68856 was used for the studies to understand determinants of peripheral virulence in mice. Virus neutralizing MAbs were generated against this strain of WN Virus. Neutralization escape mutants of peripherally pathogenic strain of WN virus 68856 selected using these MAbs have shown decreased pathogenicity. Altered reactivity and slower/decreased multiplication capacity in splenocytes and in Neuro346 cells has been observed.

Work done this year

In order to identify nucleotide changes responsible for the altered pathogenesis, sequencing of the E protein region of the parent and mutant viruses (IF1F51.2 IVC3F10 1.1 IF1A71.1) was carried out. All these mutants showed common amino acid substitutions at position 89-Alanine to Phenylalanine, 90-Phenylalanine to Leucine, 91- Valine to Leucine. However, mutant IF1A71.1 showed two amino acid substitutions at positions 156-Serine to Proline and 242-Phenylalanine to Tryptophane which were not observed in other two mutants. Of note is S 156-P substitution has been reported earlier in Flaviviruses and found to be involved in loss of neurovirulence (Table 3).

Table. 3. Amino acid substitutions in envelope protein of neutralization escape variants

Residue No	WNV 68856	Variant
50	Alanine	Valine
89	Alanine	Leucine
242	Phenylalanine	Tryptophane
156	Serine	Proline

These changes were found mainly in Domain II when mapped on 3D structure of Egp using backbone of TBE/DEN. Domain II has property of extensive rearrangement upon exposure to acidic pH (Fig. 6).

Fig. 6: Swiss Model str prediction server via Expasy and drawn with Swiss-PDB Viewer (www.expasy.ch)

Studies on the Immunoprotective mechanisms in a mouse model of JE virus

M Biswas, MM Gore

Introduction

The present study focuses on understanding cell mediated immune response in JE infection and uses an adoptive cell transfer approach to understand the process in detail in a murine system.

Objectives

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

Findings

Kinetics of JE virus infection in the brains of 15 day and 1 day old mice

Keeping in view the difficulty observed in intravenous transfer of cells in 10 day old mice as seen in the earlier model of adoptive transfer, an attempt was made to assess whether 15, 17 or 19 day old mice were susceptible to JE virus challenge by the i.p route (peripheral infection). It was seen that 15 and 17 day

old mice succumbed to the infection while no change was detected in 19 day old mice. Brains of the infected mice were harvested every 2 days post infection and amplified for a 350 bp stretch of the C-prM region of JE.(Fig. 7a) In addition, quantitation of virus was carried out using real Time PCR also in spleen , blood and brain of infected mice. (Fig. 7b)

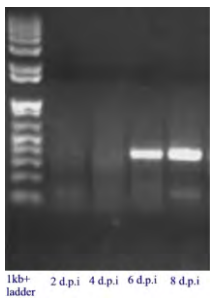


Fig. 7a: Detection of viral RNA in the brain of 2-wk-old mice infected with JEV. (350bp)

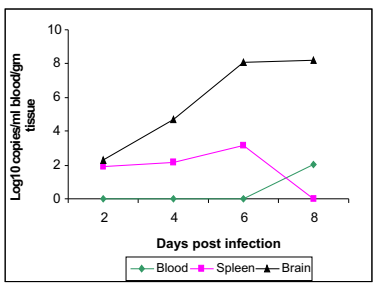


Fig. 7b: Quantitation of JE virus by Real time PCR in different organs of infected, 2 wk old mice

Detection of cytokine in different organs of 2 week old mice infected with JE virus.

In order to understand the cytokines that are synthesized upon peripheral JE virus in mice, detection of mRNA of various cytokine was carried out using RT PCR. Results showed that TNF α is generated early in brain of infected mice. IFN γ is secreted early in blood and in the later stage of infection in the brain.

Detection of cytokines in different organs of 15 day old mice infected with JE virus

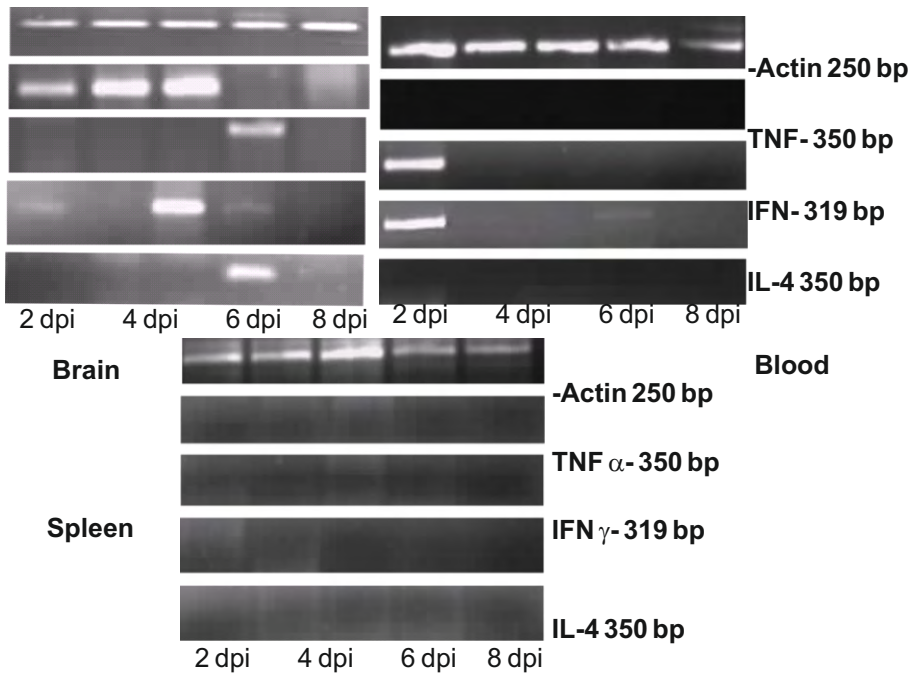


Fig. 8: Cytokine mRNA detection by PCR

Depletion of T cell Subpopulations and Adoptive Transfer into Recipient Mice

After ascertaining the suitability of 2 week old mice for peripheral challenge susceptibility, adoptive transfer of immune cells was undertaken. Upto 5 X 10^8 splenocytes from JE immunized mice were depleted of either the CD4+ or CD8+ subpopulations of T cells by magnetic cell sorting. The CD4+ and CD8+ depleted populations were stained by PE conjugated antibodies to CD4+ and CD8+ respectively and analyzed by Flow Cytometry (Fig. 9). The efficiency of depletion was seen to be 99.83% for CD4+ and 99.47% for CD8+ depleted cells.

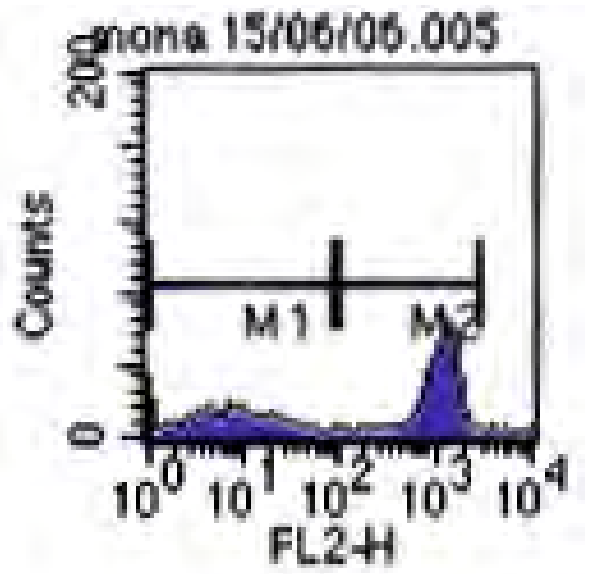


Figure 9 : Flow cytometric analysis of enriched T cell

Immune T cell either as a depleted population or enriched population were transferred. 2X10^7 cells were transferred intravenously into naïve syngenic mice. This was followed by challenge with 50 LD_{50} of JE 733913, 24 hours after transfer. Mice depleted of CD4+ splenocytes showed greater percentage mortality as compared to CD8+ T cells (Fig. 10). Similarly, mice transferred with CD8+ isolated T cells demonstrated greater mortality as compared to CD4+ T cells. It was seen that the percentage of surviving mice was greater in the CD8+ depleted splenocytes population as compared to the CD4+ depleted population (Fig. 11). This was in direct contrast to the CD8+ enriched population that exhibited greater mortality as compared to the CD4+ enriched population.

Mice transferred with primed splenocytes and those transferred with the splenocytes of unimmunized mice showed a survival percentage of 87.5 and 25 respectively (Table 4).

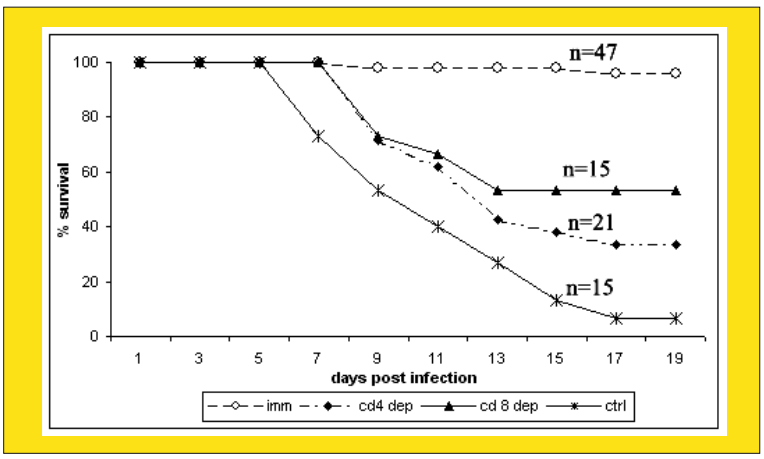


Fig.10: Effect of depletion of CD4 and CD8 subsets on protection against JE by adoptive transfer

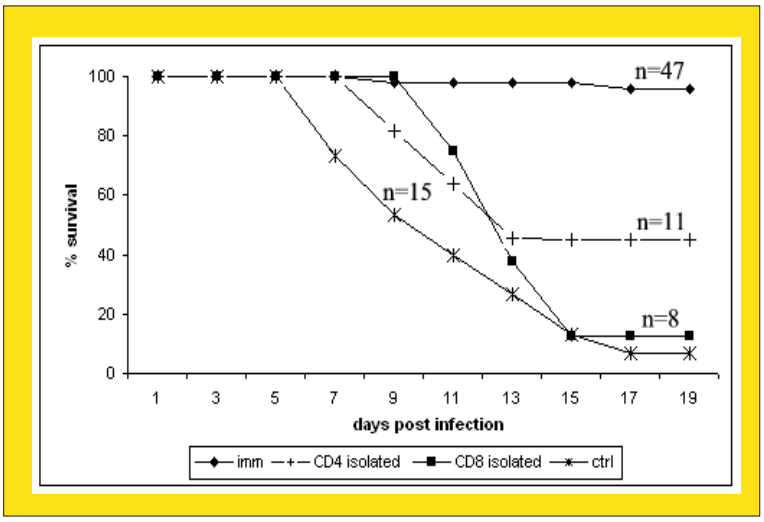


Fig. 11: Effect of adoptive transfer of enriched immune CD4 and CD8 subsets of T cells on protection against JE

Table 4: Percentage survival of mice adoptively transferred with various splenocytes subpopulations and challenged with lethal dose of JE virus

Cells Transferred	% survival
CD4+ depleted	14.28
CD8+ depleted	60
CD4+ isolated	50
CD8+ isolated	25
Primed splenocytes	87.5
Normal Splenocytes	25

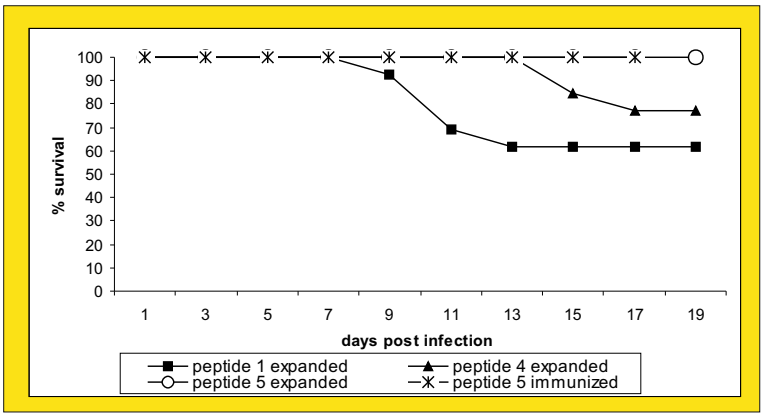


Fig. 12: Transfer of immune expanded or peptide 5 immunized cells before lethal challenge

Adoptive transfer of T helper peptide immune T cells

Immune splenocytes were also expanded in vitro in the presence of peptides with T helper epitopes of JEV. These were peptide 1 (Envelop), peptide 4 (Envelop) and peptide 5 (Membrane). Splenocytes were expanded in the presence of peptides for 7 days. This was followed by 2-3 cycles of restimulation in the presence of fresh APC's and recombinant IL-2 in addition to the peptides. As peptide 5 stimulated cells provided complete protection upon transfer and challenge with JE, a fresh set of mice were immunized with 3 doses of peptide 5 in alum. This was followed by transfer of the peptide 5 immunized splenocytes and challenge. It was seen that peptide 5 alone could provide complete protection against JEV (Fig. 12).

Behavioral and pathological effect of Indian isolate of West Nile Virus

Raut C.G.

Introduction

The major focus of this research project was aimed in understanding the effect of WNV in hamsters as a model of infection.

Objectives

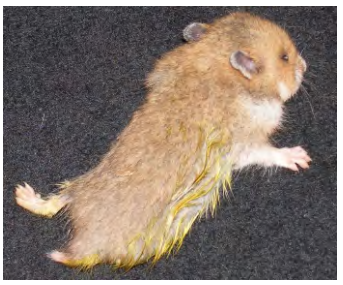
To develop an animal model for WNV infection

Findings

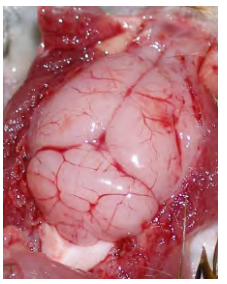
Subcutaneous infection with WNV showed the virus tropism to the brain on 2nd day except 4 week old animals, wherein it was on 3rd day in both brain and spinal

cord, as detected both immunohistochemically and histologically. Brainstem was the primary and consistent target, from which the virus sequentially reached to pons, cerebral cortex and Purkinje cells of the cerebellum from 4th day onwards. Neuronal degeneration started initially on 2nd day in 1 week old animals, 3rd day in 2nd and 3rd week old animals and 4th day in case of 4 week old animals, showing a corresponding gradation in the susceptibility of these experimental animals. The antigen was demonstrated in spinal cord on 3rd day in case of 1 and 2 week old animals, whereas it was only on 4th day in both 3 and 4 week old animals.

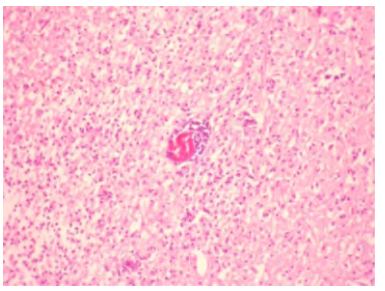
Apoptosis could be detected in the infected cells in all the age groups from the 4th PID. The animals remained normal till this stage. From the 5th PID onwards, the virus was present in almost every part of the brain and spinal cord that coincided with pathologic lesions. During this stage, the animals started showing behavioural changes such as huddling, somnolence, decreased response to the surroundings, ruffled fur, signs of limb weakness, exhibited by sluggish mobility, inability to stand etc. It was on the 6th day, the animals showed characteristic neurological symptoms like reduced righting reflex, reduced ability of hindlimb conscious proprioception, reduced grip strength of forelimbs and reduced reflexes of pinna, cornea and toe. This was coincided by increased severity of lesions in brain and spinal cord like gliosis, intensive Perivascular cuffing and accumulation of mononuclear cells suggesting lymphoplasmacytic encephalitis.During these stages, the animals showed signs like tilting of head, paddling of legs, circling movements, shaking of head, twitching of tail and paralysis. Apoptosis was detected minimally after 7th day and on the 8th day, was found focally in the brainstem area only. Among the other visceral organs except spleen, neither lesions nor the antigen could be detected, which suggests a higher predilection and rapid action of this virus towards CNS. But WNV also possesses immunosuppressive action as substantiated by progressive depletion and destruction of lymphocytic population in spleen. As a whole, the study indicates a higher susceptibility of younger animals to WNV infection of Indian isolate G22886.



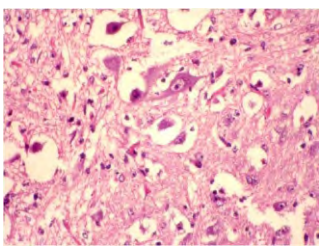
1) In-coordination of limbs



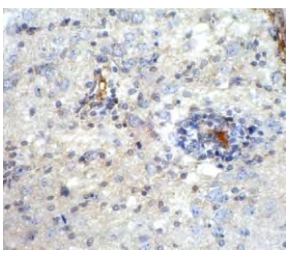
2) Congestion of meningeal vessels



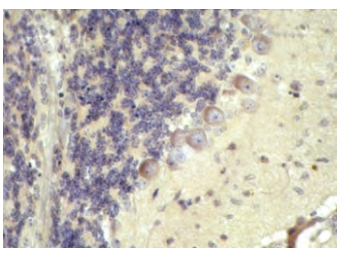
3) Perivascular cuffing in brain leading to paralysis



4) Spongiosis in brain



5) IHC positive Nueron within glial nodule



6) IHC positive PurkinJE cells

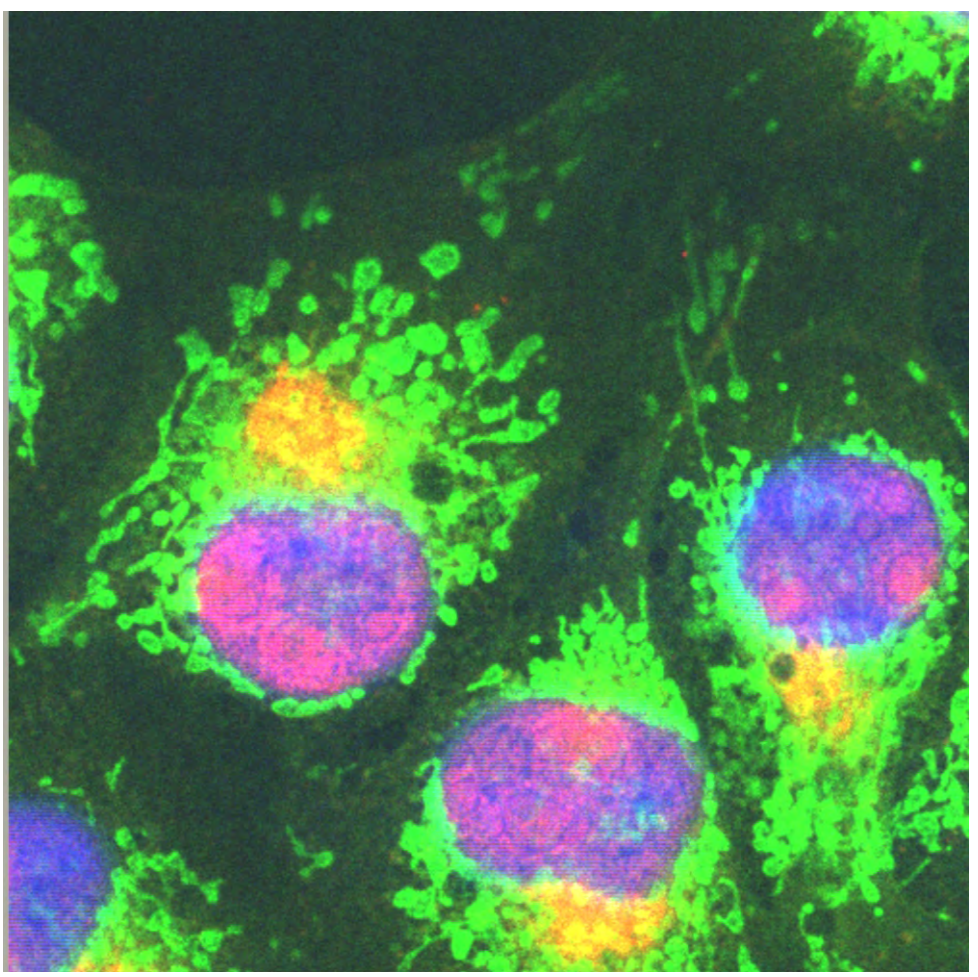
Fig 13: Representative figures of pathobiological changes occurred in said age groups of animals

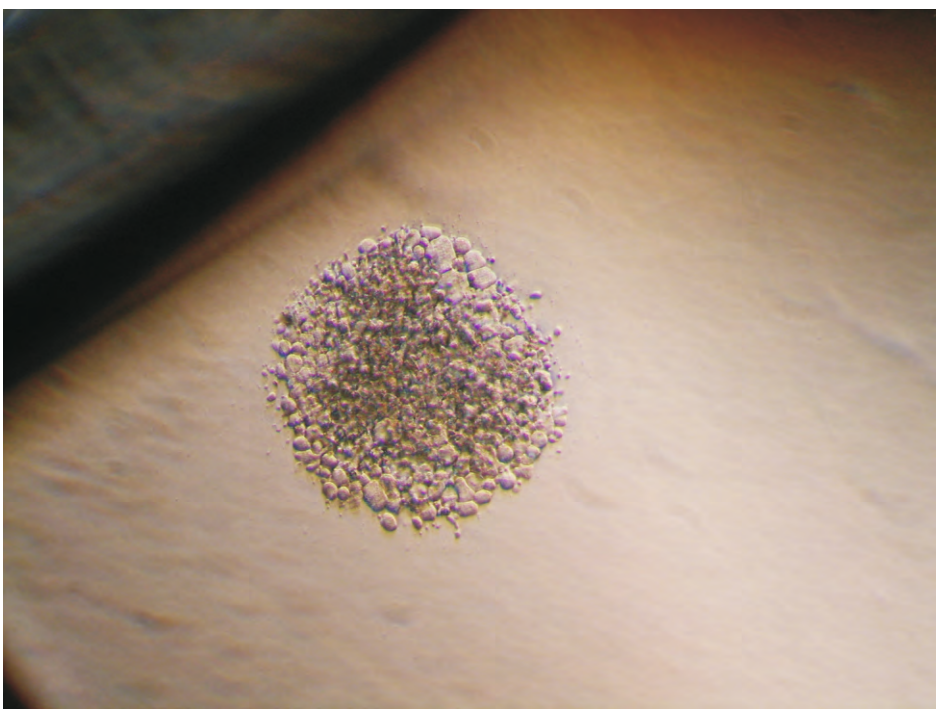
Publications

1 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, 88: 875-84.

2 Devasthaly SS, Bonde GS, Shankarraman V, Biswas SM, Ayachit VM, Gore MM (2007). Chimeric T helper-B cell peptides induce protective response against Japanese encephalitis virus in mice. Protein and Peptide letters 14: 543-51.

Dengue Virus





A CFU-MK colony differentiated in vitro from human CD34+ cells

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Determination of disease burden for dengue

Cecilia Dayaraj, Anand Singh, Priyadarshini D, Dr.Paresh Shah

Introduction

Determination of the cost of dengue to the country is very important from the public health point of view. In the previous year we had reported our investigations on dengue in Pune in 2005 with reference to the clinical features, the demographic data, the ratio of primary to secondary infections and virus serotypes circulating in the city. Similar investigations carried out over a period of five years would help us understand the disease pattern. The present report extends the data for 2006-07.

Work done

The incidence of dengue in Pune during 2006 was very low. A total of 65 samples were collected from suspected dengue cases admitted to the KEM hospital in Pune during July-October. Of these 27/65 (41.5%) were positive for dengue-specific IgM antibodies. The cases could be classified according to WHO criteria into 14 DF (52%) and 13 (48%) DHF cases. The DHF cases could be further divided into 4/13 DHF-I and 9/13 as DHF II grade. The larger number of DHF cases was probably due to sampling from only hospitalized cases. Samples were received from Maharashtra, Gujarat and Kerala for detection of dengue-specific IgM antibodies during the period October 06-March 07. From Maharashtra, 142 samples were received. The total percentage positivity was 12% with Akola reporting highest positivity (42%) followed by Mumbai (27%) and Sangli (15%). None of the samples received from Gujarat and Kerala showed presence of IgM antibodies specific to dengue virus. Our efforts during the year were focused on developing tests required to carry out sero-surveys and studies on prevalence and incidence of dengue in the city.

Standardization of MAC-ELISA with cell culture based virus

Several Indian strains of each serotype were passed in Vero cells to get cell culture derived antigen for ELISA. The Indian strains of three serotypes DENV-2 (803347), DENV-3 (058926) and DENV-4 (642069) were successfully adapted to Vero cells. Adaptation of DENV-1 is ongoing. The cell culture adapted DENV-2, 3 and 4 viruses were compared in the MAC-ELISA test with the DENV-2 mouse brain derived antigen that is currently being used. The results obtained were equivalent. The MAC-ELISA was further modified to a 3-1/2 hr test. Patient sample was added to anti-IgM pre-coated wells. The presence of dengue specific IgM was detected by addition of precomplexed virus + anti-dengue MAb-

biotin conjugate mixture. The titration curves of a control dengue immune serum with cell culture derived virus (single or mixed serotypes) were similar to that seen with DENV-2 mouse brain derived antigen (Fig.1).

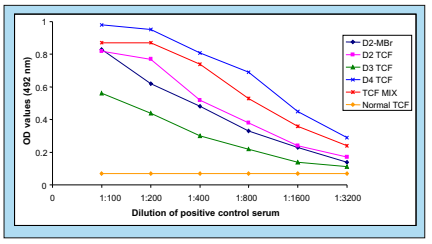


Fig. 1: Titration curves of a dengue IgM-positive serum against the different cell culture based and mouse brain derived antigens

Standardization of IgG-ELISA

The IgG-ELISA had to be standardized for the sero-survey study and for determination of primary/secondary infections. The dengue-specific monoclonal antibody, E3F6 (courtesy G N Sapkal, JE group) was used to capture cell culture derived antigen. Test samples were added and the IgG captured was detected with anti-Human IgG HRP conjugate. The results with two pairs of control positive and negative sera, one from Thailand and one from the lab are shown below in Table1.

Table 1: IgG capture ELISA using cell culture based DENV-2 antigen

Sample	OD value	P/N ratio*	S/N ratio@
Positive Thai	1-5±0.34	4-4	4.96
Negative Thai	0.3±0.1	-	1.5
Positive Lab	1.3±0.32	4-6	5.01
Negative Lab	0.2±0.04	-	1.27

*OD of sample/OD of negative serum
@ OD of sample with DENV-2 antigen/OD of sample with normal antigen

Standardization of Plaque Reduction Neutralization test (PRNT)

The plaque reduction neutralization test needs to be developed against all four serotypes. Indian strains representing all four serotypes were taken from the virus repository and adapted to PS cell cultures. After 4-5 passages the virus strains were tested for their ability to form plaques. Adaptation of DENV-2, 3 and 4 strains to PS cells was achieved and the viruses were shown to form plaques. The PRNT test was standardized against the DENV-2 strain (803347) in 24 well plates. Plaques could be seen by 4-5th post infection day.

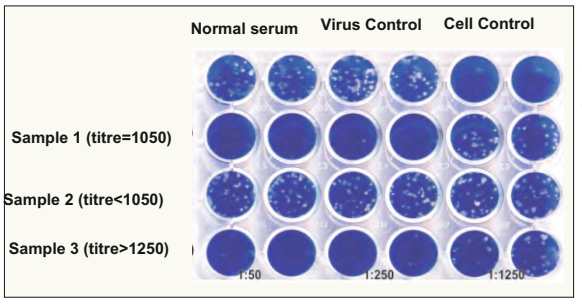


Fig. 2: Plate showing PRNT test against DENV-2 803347

Evolution of dengue viruses in the country

JA Pawar, Cecilia D

Introduction

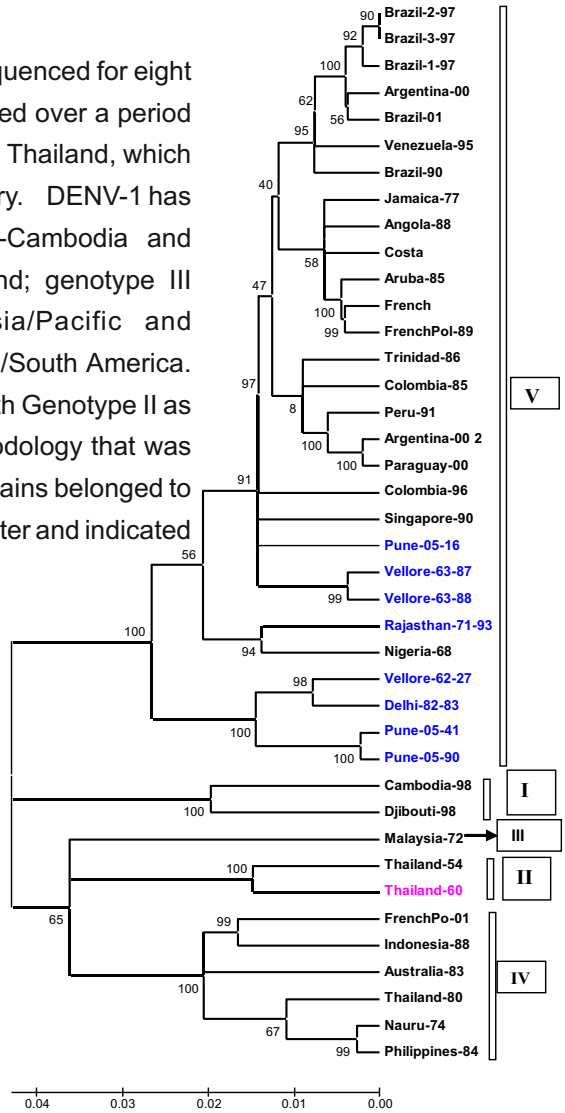
Phylogenetic analysis of the dengue viruses circulating in India is being pursued since 2003 to understand the evolution of dengue viruses in the country. We had earlier reported the switch in genotypes of DENV-2 viruses during the 1980s. Phylogenetic analysis of the other serotypes and additional strains of DENV-2 was undertaken during the past year. All the phylogenetic analyses were carried out using MEGA-3. The trees were constructed with NJ method (1000 replicons).

Findings

DENV-1

The envelope (E) gene was sequenced for eight Indian strains of DENV-1 isolated over a period of 50 years and one strain from Thailand, which was present in the NIV repository. DENV-1 has five genotypes, genotype I -Cambodia and Djibouti; genotype II - Thailand; genotype III Malaysia; genotype IV Asia/Pacific and genotype V Caribbean region/South America. The Thailand strain grouped with Genotype II as expected supporting the methodology that was being followed. All the Indian stains belonged to genotype V, formed a loose cluster and indicated multiple introductions.

Fig. 3

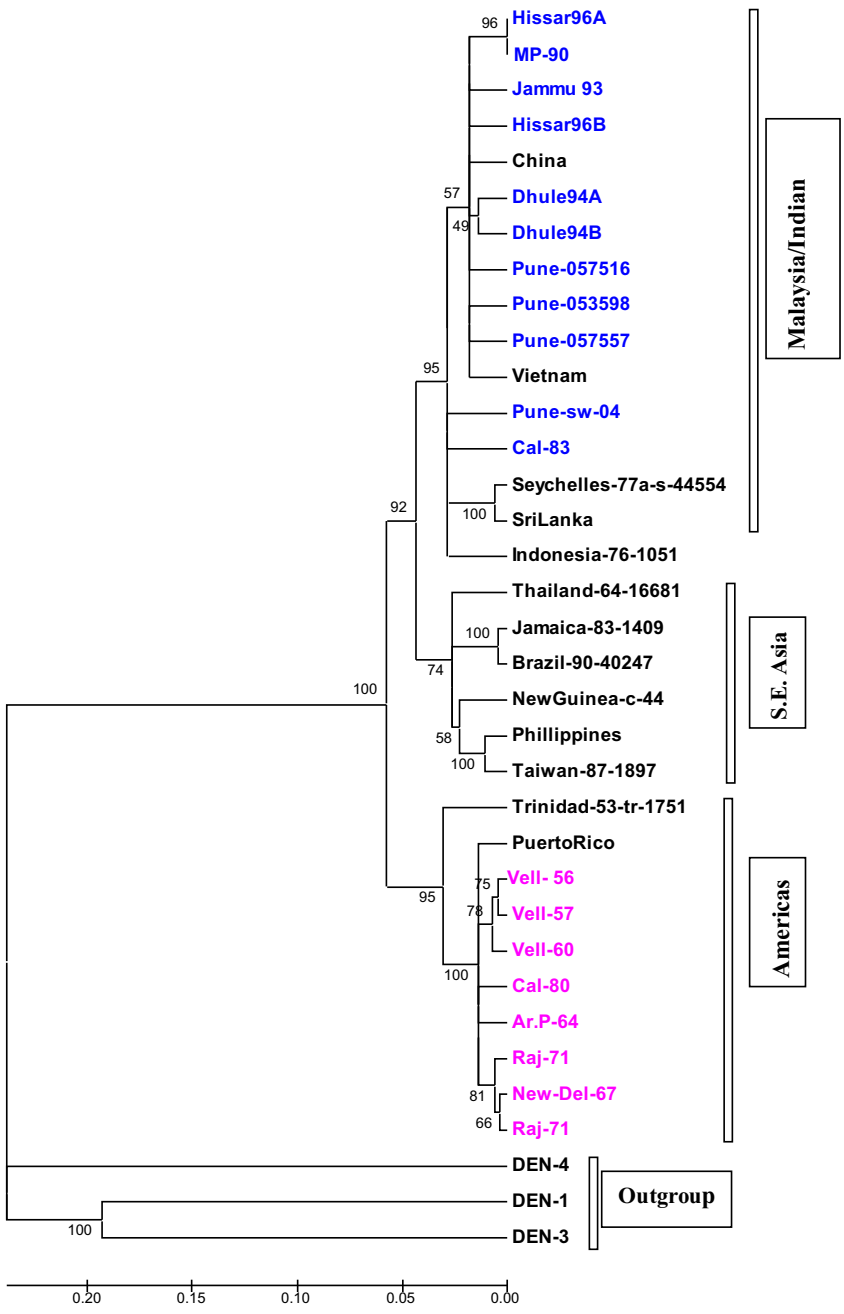


High bootstrap values (70100%) indicate robust support for the tree

DENV-2

Earlier we reported a genotypic shift from American genotype to Malaysia/Indian genotype in the 1980s. We had used 1500 nt sequence of E gene of 10 strains for this study. Subsequently we sequenced 11 more strains and found the pattern remained the same using the data of all the 21 strains. Strains before 1990 belonged to the American genotype and after 1990 the strains belonged to the Malaysia/Indian genotype.

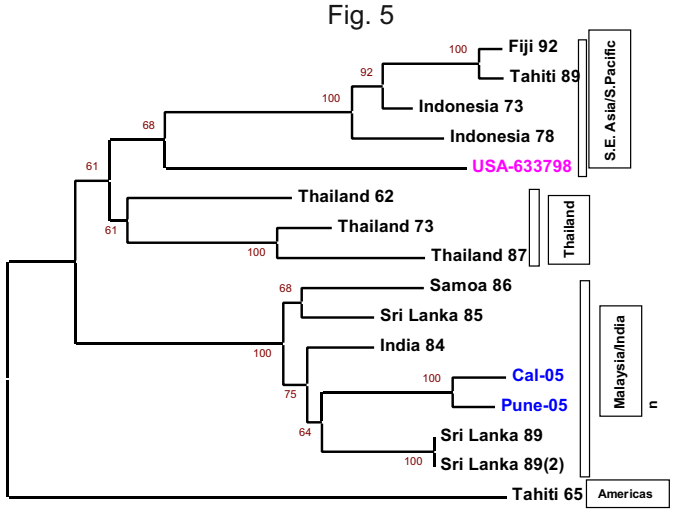
Fig. 4



DENV-3

Based on E gene sequence, earlier reports have classified DENV-3 strains into four genotypes, the SE Asian/Pacific, the Thailand, Malaysian/Indian and the American. We sequenced the E gene of four strains, one of these was deposited by Rockefeller Foundation, USA. All Indian strains belonged to Malaysian/Indian subcontinent genotype whereas the USA strain clubbed with the Southeast Asia Pacific genotype

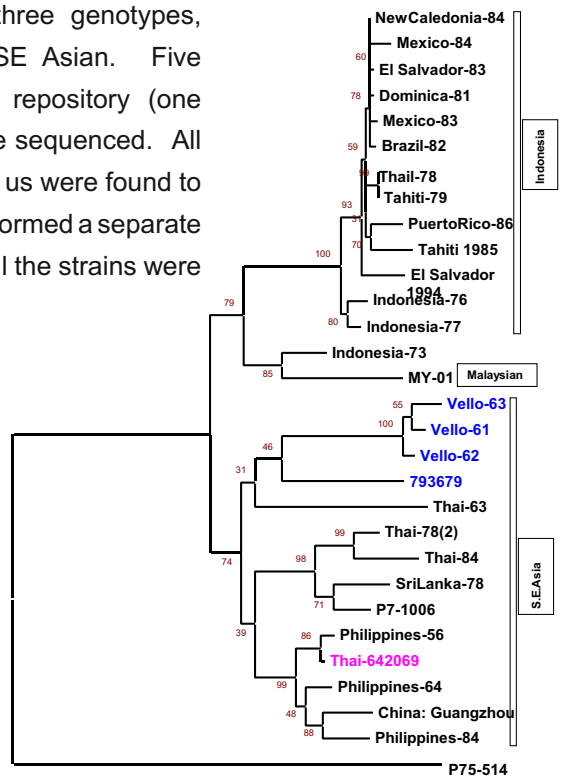
Fig. 5



DENV-4

Fig. 6

The DEN-4 serotype has three genotypes, Indonesian, Malaysian and SE Asian. Five isolates present in the NIV repository (one originated from Thailand) were sequenced. All the five isolates sequenced by us were found to be the SE Asian genotype and formed a separate cluster within the genotype. All the strains were isolated before 1980.



Future plans: the loose clustering of DENV-1 isolates in genotype V will be further analyzed in context to the genome's stability. DENV-2 has shown a genotype switch and the full genome will be further analyzed for better understanding of the switch.

Additional strains of DENV-3 are being sequenced. DENV-4 dos not seen to be in circulation hence the analysis will be restricted to the older strains.

The role of host/viral factors in dengue immunopathogenesis

Priyadarshini D, Guru Kumar K R, Rupali Bacha, Cecilia D

Introduction

Both host and virus factors are important in the development of severe dengue disease. We have been investigating the role of the host immune response and virus load in dengue patients. We have already reported the significance of IFN-gamma, IL-6 and IL-8 in dengue. During the current year the humoral response and the viral load were assessed in dengue patients.

Work done

Antibody response in Dengue patients

Neutralizing antibody (NAb) titres were assessed in 131 individuals by plaque reduction neutralization test against an Indian strain of DENV-2. The NAb titres were compared in DF and DHF patients. All the DHF (32) cases, except one, showed the presence of NAb, 22% had titres less than 1250 and 75% of them had titres greater than 1250. On the other hand 16% of DF (99) cases had no detectable Nab, 29% had titres below 1250 and 55% had titres more than 1250. The high titres of NAb were indicative of secondary infection and showed a stronger association with the occurrence of DHF.

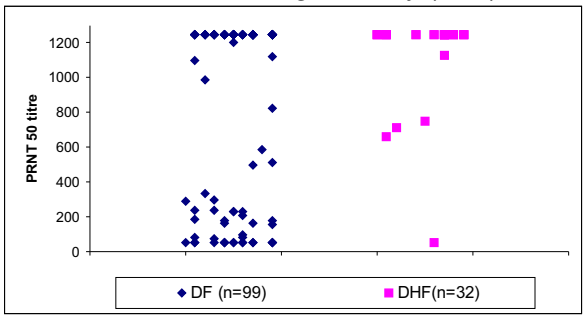


Fig. 9: DENV-2 neutralizing antibody titres in dengue patients

Viral load in dengue patients

A group-specific real time PCR assay was developed for the quantitation of viral RNA in patient sera. The 3' terminal 100 nucleotides of the genome, conserved across the serotypes, was selected to develop Real-time primers and Taqman MGB probes. The region amplified by the primers developed for real time PCR was 67bp long. The PCR amplified product from a DENV-3 strain was cloned into the TA vector. The RNA was reverse transcribed off the cloned 67 bp region, using the reverse primer and SP6 polymerase. The product was used in the Real

time assay to determine the sensitivity of the assay. The system could detect from 100 copies to 10^8 copies of RNA/reaction. A total of 340 sera samples from dengue suspected cases were tested in the real time assay and 59 were found to be positive for dengue virus RNA. The viral load in dengue patients ranged from 10^4 to 10^{13} RNA copies (Fig 8a). The mean viremia in DF cases was $4.75x10^{11}$ while in DHF cases it was $7.65x10^{11}$. Viremia was higher during the early phase of infection in both DF and DHF cases and then rapidly reduced from day five onwards. In the later stages viremia was found to be higher in DHF cases though more sampling of DHF cases is required to statistically validate the data (Fig 8b).

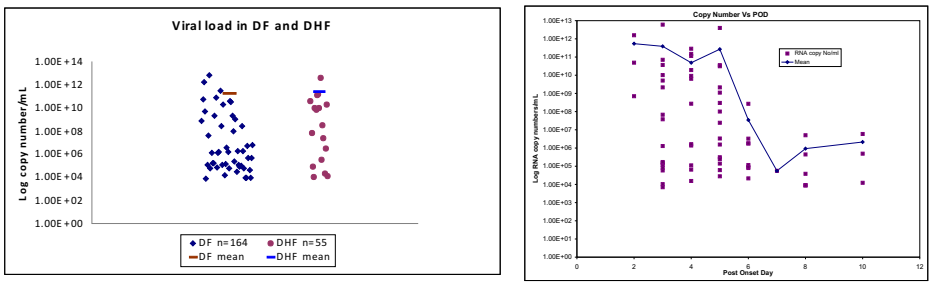


Fig. 10: The virus load was determined in the sera of DF and DHF patients by real time PCR, the virus load has been shown as RNA copy numbers in (a) and the viraemia in relation to the post onset day of sample collection is shown in (b)

Interactions of dengue virus with Interaction of dengue viruses with cellular organelles during viral morphogenesis

Samatha Sripada, Nidhi Srivastav, Cecilia D

Introduction

Our earlier studies on dengue morphogenesis had revealed the role of actin and microtubules in dengue entry and exit. During the past year the studies were extended to the intermediate filaments (IF).

Work done

Cells infected with DENV-2 were labeled for IF. Double staining of vimentin and DENV-2 at various time points revealed changes only during the later phase of infection. Late in infection, before cell lysis, the IF structure collapsed and only a perinuclear aggregate could be observed which harboured virus (Fig.11).

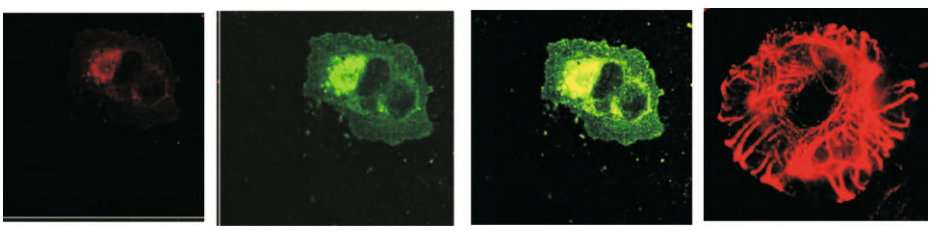


Fig. 11: Effect of DENV-2 infection on IF structure - the IF is labeled red and the virus is labeled green, a) perinuclear IF, b) dengue antigen, c) Colocalization of IF and dengue antigen, d)IF structure of uninfected cell.

To study the interactions of individual viral proteins with cellular proteins, the prM-E and NS1 genes of DENV-2 were cloned into a mammalian expression system. The authenticity of the expressed proteins was confirmed by testing its antigenicity and immunogenicity. BHK-21 cells transfected with the recombinant plasmids were shown to express the viral proteins. The recombinant prM-E and NS1 expressed in the transfected cells could be recognized by anti-dengue antibodies in the immunofluorescence assay. When inoculated into mice by the i.d. route, the recombinant plasmids induced an antibody response in the inoculated mice. It was noteworthy that mice immunized with 2 doses of pVaxD2preM-E alone had antibody levels equivalent to mice given a single dose of virus. Furthermore, inoculation of pVaxD2preM-E prior to live virus resulted in boosting the antibody response. PVaxD2NS1 also induced antibodies in inoculated mice but very low levels were observed. The results proved the authenticity of the expressed proteins. The cloning of other genes and the interactions of the expressed proteins with cellular proteins is ongoing.

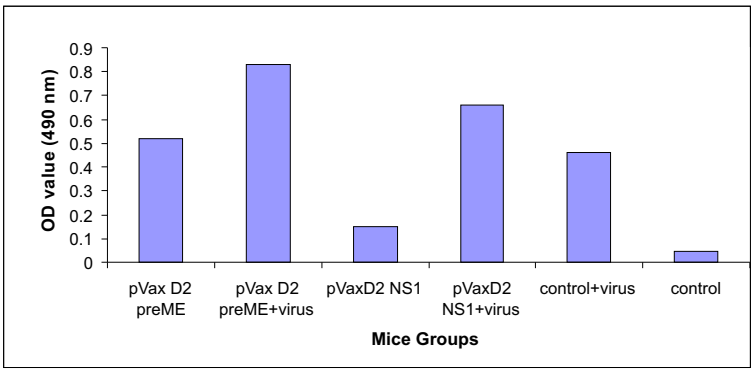


Fig.12: The antibody response detected in mice immunized with recombinant DNA. Sera from four mice per group was pooled and tested for anti-dengue antibodies in ELISA at a dilution of 1:50.

siRNAs an antiviral tool against dengue

Paresh Shah, Guru Kumar K R, Cecilia D .

Introduction

shRNA as an antiviral against dengue has the advantage of a single agent being effective against all four serotypes. The selection of a region in the 3'UTR common to all four serotypes and the designing of shRNA was reported last year. Preliminary in vitro studies with the shRNA clone had shown marginal decrease in virus replication in cells transfected with the DEN-shRNA prior to infection with the virus.

Work done

Further studies on optimization of treatment as regards transfection reagent, dose and time were undertaken. The optimization studies were carried out with the reporter gene, EGFP (Enhanced Green Fluorescent Protein). Transfection reagent from three manufacturers was used for optimization and siPort (Ambion) at the concentration 10ul with pEGFP at a concentration of 1 ug/ml was found optimum. Maximum fluorescence was observed at 96 h post transfection.To assess the effect of shRNA on viral RNA, BHK-21 cells were transfected with different concentrations of DEN-shRNA plasmid prior to being infected with DENV-2 virus. The transfected cells were infected with two concentrations of DENV-2, 0.01 and 0.1 MOI. The effect of shRNA on dengue virus replication was assessed by quantitation of viral RNA in the infected cell culture supernatant by real time PCR. The DEN-shRNA inhibited DENV-2 virus replication at a concentration of 40ng. The viral RNA in supernatant of transfected cells was less than the viral RNA present in non-transfected cells by 2 logs. Further experimentation with stably transfected cells is ongoing.

Interaction of Dengue viruses with platelets

Preksha Jain, Shobha Gangodkar, Kanjaksha Ghosh *, Atanu Basu
Institute of Immunohematology, Parel Mumbai *

Introduction

Thrombocytopenia and hemostatic dysfunctions are frequently associated complications of dengue disease. Although several factors, mostly immunopathogenic host responses have been implicated in the origin of thrombocytopenia, the role played by dengue viruses per se in interacting with platelets remain incompletely understood. Previous studies in our laboratory had shown selective loss of collagen induced aggregation responses of platelets

after exposure to dengue 2 virus. We further studied this interaction at several levels.

Objectives

Characterize the interaction between dengue 2 virus and normal platelets using Atomic Force and electron microscopy

Summary of findings

In the present study we examined the effect of dengue 2 virus on the morphology and physiological activation profile of normal human platelets using atomic force microscopy, electron microscopy and flowcytomtery. Platelets obtained from healthy donors were exposed to a cell culture adapted 10^4 LD_{50} dose of a dengue 2 virus isolate in-vitro and its subsequent effect on morphology and activation biology studied. Our results show that dengue 2 virus exposure at doses comparable to natural viremic states in human infections can activate platelets with increase in P-selectin expression and fibrinogen binding property. Atomic force and scanning electron microscopy studies also showed activation-related morphological changes in platelet membrane architecture while ultrastructural changes like degranulation, presence of filopodia and dilatation of the open canalicular system (OCS) were observed by transmission electron microscopy of dengue 2 virus exposed platelets. Importantly, Japanese Encephalitis Virus (JEV) exposure at the same dose as dengue 2 virus did not activate platelets or show any morphological changes. Our findings indicate that direct activation of platelets by dengue 2 virus might be a key feature in the origin of dengue-associated thrombocytopenia. Detailed molecular characterization of this effect might provide key knowledge towards better prophylaxis of the hemostatic complications of dengue disease.

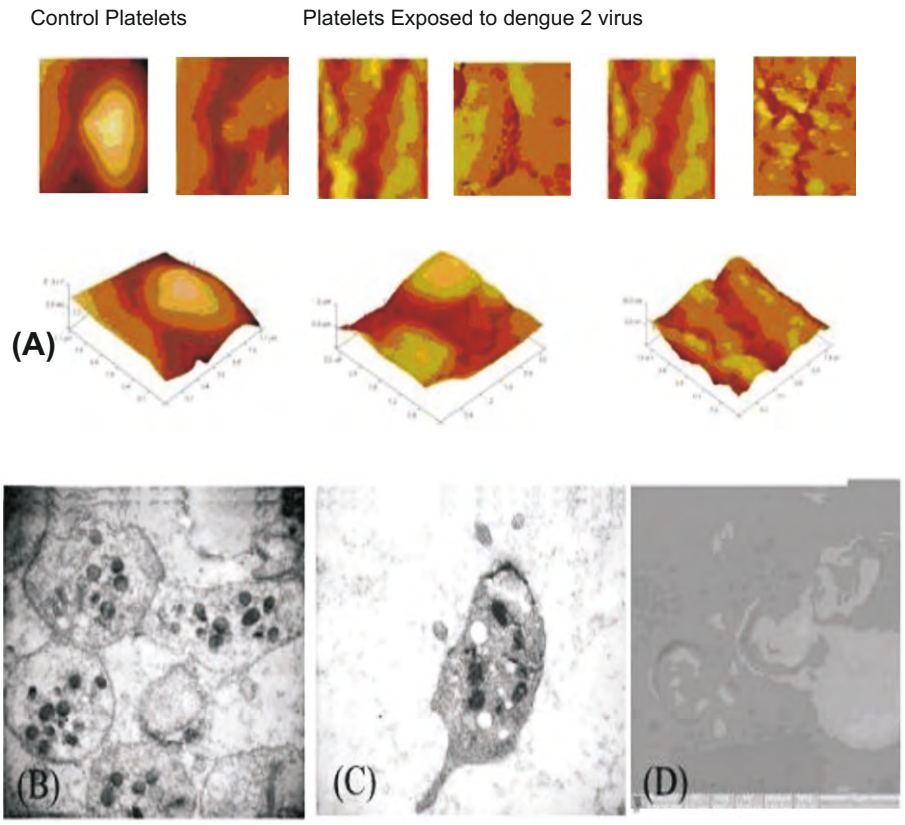


Fig. 13: Imaging the fine structural alterations in platelet membrane surface and ultrastructure after exposure to dengue 2 virus. (A) This panel shows representative atomic force microscopy images of the platelet surface in control and dengue virus exposed platelets. The bottom row of AFM images are height reconstructions of the force contours showing the alterations in platelet membrane topography after exposure to dengue 2 virus (B) A representative Transmission Electron micrograph of a platelet showing normal ultrastructure and presence of granules (C) representative electron micrograph of a platelet after dengue 2 virus exposure showing typical “activation” ultrastructure (D) a low power ESEM micrograph of dengue 2 virus exposed platelet field showing discoid to spherical transition after dengue 2 virus exposure

Effect of dengue viruses on in-vitro hematopoiesis

Preksha Jain, Shobha Gangodkar, Kanjaksha Ghosh *, Atanu Basu
Institute of Immunohematology *
Extramural funding: Dept of Biotechnology, Govt of India, 2005-2008
Total: Rs 46.95 lakhs

Summary of work

Highly purified umbilical cord blood derived CD34+ cells were differentiated in the presence of thrombopoietin (TPO) and thrombopoiesis specific growth factors in-vitro and effect of dengue 2 virus isolates studied using both in-vitro clonogenic assays for megakryocyte colony forming units (CFU-Mk) and in liquid cultures. Significant inhibition of the CFU-Mk by dengue 2 viruses in a dose dependent manner and not seen by heat-killed dengue 2 virus and Japanese Encephalitis Virus (JEV). In liquid cultures, there was evidence of significant loss of cells in culture by the 5th day of virus exposure that corresponded with presence of committed megakaryocytic progenitor cells as seen from antigenic phenotyping data for CD34+CD61+ cells. Moreover, this window of cell loss also coincided with high expression of annexin V and PI binding suggesting enhanced apoptosis in CD34+ committed progenitors exposed to dengue 2 virus. Current studies are ongoing to characterize this effect in more details.

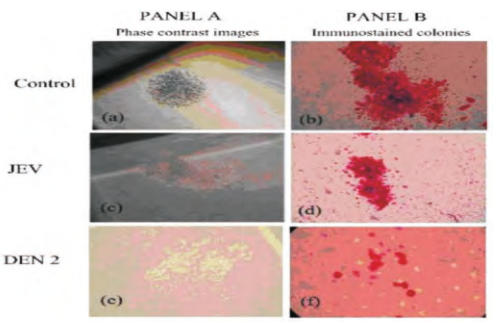


Fig. 14: Effect of dengue 2 viruses on CFU-Mk of CD34+ cells in-vitro
(a) control (b) immunostained CFU-MK (c-d) CFU-Mk after JEV exposure (e-f) CFU-MK after dengue 2 virus exposure

Characterization of the Dengue virus induced cytotoxic factor

Atanu Basu, UC Chaturvedi

Dengue virus induces a cytotoxic factor with role in disease pathogenesis in both natural and experimental infections, as shown earlier. Under the guidance of Prof UC Chaturvedi, a new project was developed to characterize this CF further and evaluate its possible prognostic role in dengue infections. Preliminary studies have been initiated.

A Bioinformatics approach to study the interaction between dengue viruses and blood platelets

Dr Sarah Cherian, Atanu Basu

Introduction

Earlier studies (NIV unpublished data) had suggested that exposure to dengue 2 virus leads to the loss of collagen agonist effect on blood platelets. In order to understand further the possible molecular basis of this interaction, the following study was planned using bioinformatics approach.

Objectives

Identify possible dengue 2 virus binding molecules on platelets and use of bioinformatics tools to understand the molecular basis of dengue virus interaction with blood platelets.

Work Done

Preliminary modeling and homology search results suggests that the dengue viral E protein might have shared motifs for metalloproteinase binding with snake venom toxins which are also hemorrhagic. Further studies are ongoing.

Participation in meetings, workshops

Dr. (Ms) Cecilia D

Invited speaker at the "41st Japanese encephalitis Virus Ecology Study Meeting" held at Nagasaki, Japan from 24th to 28th May 2006.

Meeting with Honorable Health Minister of State, Kerala and the Secretary (Health and Family Welfare, Government of Kerala), Dr Dinesh Arora, State Mission Director, NRHM at Trivanderum, Kerala on the 17th March 2007 to discuss the establishment of a field station at Alappuzha, Kerala.

Dr. PS Shah

The meeting to discuss a proposal entitled "A pilot study to evaluate the safety and preliminary efficacy of the hemopurifier for the treatment of HIV in subjects with end stage renal disease requiring dialysis" submitted by Aethlon, USA and Qualtran, India .The meeting was held at ICMR head quarters on 21, February 2006

The meeting to evaluate the proposal "Use of hemopurifier as a treatment for dengue hemorrhagic fever". The meeting was held at ICMR head quarters on 22, September 2006.

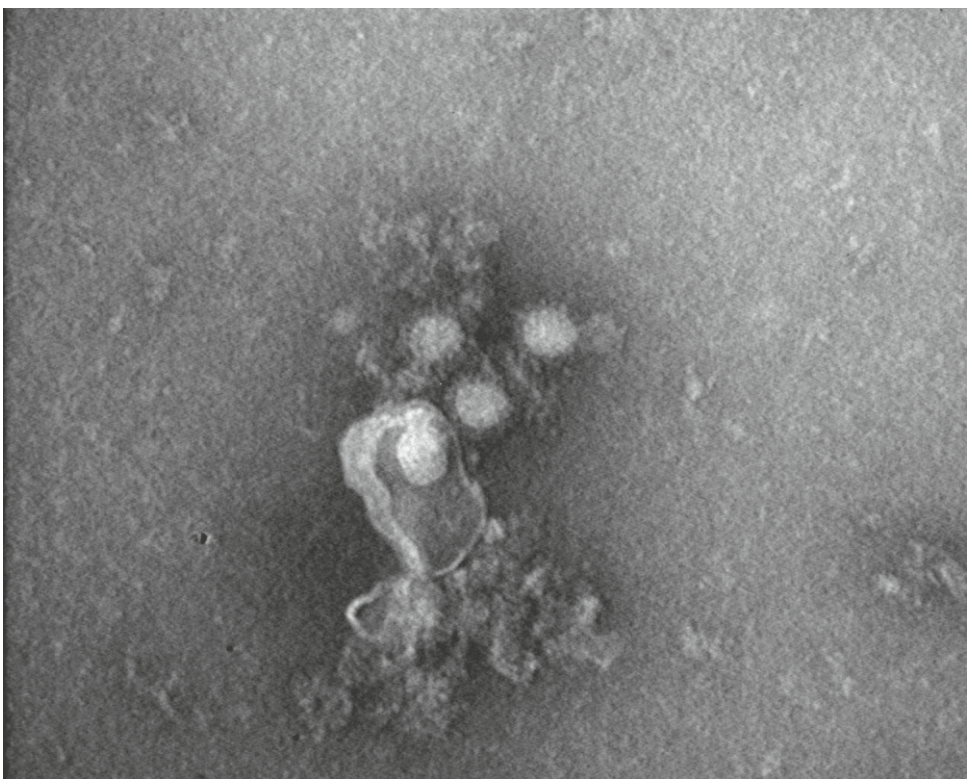
Delivered a lecture as a invited guest speaker at a symposium on Dengue organized by "Association of Physicians Of India." On 31st October 2006.

The meeting with Honorable Health Minister of State, Kerala and the Secretary (Health and Family Welfare, Government of Kerala), Dr Dinesh Arora, State Mission Director, NRHM at Trivanderum, Kerala on the 17th March 2007 to discuss the establishment of a field station at Alappuzha, Kerala.

Group Participation

The Dengue Group participated in Asia Pacific Conference on Medical Virology, New Delhi, Novermber 13-15, 2006.

Chikungunya Virus



Chikungunya Virus Group

Scientific Staff

Dr. VA Arankalle, Deputy Director (Sr. Gr.), Group Leader
Dr. Deepti Parashar

Research Fellows

Manish Singh

Serology of Chikungunya (CHIK)

Sathe PS

Between 2006-07, 20,0000 sera samples collected from patients with febrile arthralgia were tested for presence of chikungunya virus specific IgM antibodies using kits developed in-house. The results are presented in Table No 1

Table 1: Distribution of IgM antibodies to CHIK

State	Total Districts affected	Samples Tested	CHIK IgM +ve
Maharashtra	32	4465	1697
Karnataka	20	767	247
Tamil Nadu	25	1077	469
Andhra Pradesh	21	665	277
Gujarat	12	459	167
Rajasthan	3	99	28
Madhya Pradesh	4	87	42
Orissa	1	34	6
Uttar Pradesh	2	33	26
Kerala	10	77	41
Union Territories	4	114	11
Total	-	7877	3011

Complications of Chikungunya virus infections

Neurological complication was observed in 182 patients admitted to various hospitals in Maharashtra. Most of the patients were admitted with altered sensorium. Serum/CSF collected from these patients were tested for presence of Chikungunya virus specific IgM. Sixty-seven serum/CSF pairs (38%) had detectable levels of virus specific IgM. In one case, both the CSF and corresponding serum were positive for Chikungunya IgM.Opthalmic complications were mainly observed as optic neuritis and uveitis. There was a positive correlation between Chikungunya attack and the occular complications.

Detection of CHIK virus IgM antibodies in human serum samples using head squashes of CHIK infected *Aedes aegypti* mosquitoes

Mourya DT, Sudeep AB

A simple and rapid method was established for detecting CHIK virus antibodies using head squashes of CHIK infected *Aedes aegypti* mosquitoes as immobilized antigen. The sensitivity and specificity of the test was evaluated with

MAC-ELISA and C6/36 cells in detecting the presence of CHIK antibodies (Table 2).

Table 2: IgM antibodies detection in sera samples using mosquito head squash technique, C6/36 cells and ELISA

	ELISA tested samples	
	OD P/N; >2 [n=20]	OD P/N; <2 [n=20]
%	100	100
	Mosquito head tissues test	
%	80	100
	% ELISA positive samples undetected by mosquito head tissues test	
%	20*	0
	C6/36 infected cells test	
%	80	100
	% ELISA positive samples undetected by C6/36 infected cells test	
%	20*	0

*= Borderline positive samples in MAC ELISA

Chikungunya virus isolation from Clinical samples

Sudeep AB

A total of more than 200 clinical samples for were processed Chikungunya virus isolation and 58 strains were isolated. Out of these, 52 strains were from serum, 2 were from CFS and 4 were from mosquito pools.

Molecular Characterization of Chikungunya virus isolates from India

Arankalle VA

The NIV was the first laboratory to identify Chikungunya infections in the country; initially in Karnataka followed almost simultaneously in Andhra Pradesh and Maharashtra leading to the declaration of the epidemics of the disease by the respective state governments. Special investigations were undertaken in these states. A patient with acute onset of moderate to high-grade fever with joint pains of varying severity, negative for malaria and other common causes of illness and positive for IgM antibodies to CHIK virus (IgM-anti-CHIK) seroconversion to anti-CHIK antibodies / CHIK virus isolation was confirmed as Chikungunya case. The cases were reported predominantly from rural areas with focal distribution. Multiple members in families were affected. All ages and both sexes were affected with more cases in persons aged fifteen years and above (299/333, 89.8%, $p<0.001$). Eleven of 23 districts in Andhra Pradesh, 15 of 27 in Karnataka, 16 of 35 in Maharashtra were affected.

In Andhra Pradesh, IgM-anti CHIK and IgM-anti-Dengue antibodies were detected in 251/565 (44.4%) and 17/325(5.2%) cases whereas both IgM antibodies were recorded in 14 of 325 (4%) cases. Of the 33 sera processed in C6/36 cell line, 20 yielded CHIK virus isolations. CHIK virus was detected in 8 of 12 pools of Ae. aegypti females.

In Karnataka, 303/900 (33.7%) cases were positive for IgM-anti-CHIK and 20/191 (10.5%) for IgM-anti-Dengue antibodies. CHIK virus was isolated from 83/93 (89.2%) sera and four Ae. aegypti females.

In Maharashtra, IgM-anti-CHIK antibodies were detected in 169/473 (35.7%) whereas IgM-anti-Dengue antibodies were present in 25/473 (5.3%) cases. All 9 sera inoculated in C6/36 cell line yielded the virus. IFA detected CHIK antigen in 11/35 Ae. aegypti females.

The population of Aedes aegypti was reasonably high in most of the localities; in Andhra Pradesh, AHI and BI ranged from 10-60 and BI ranged from 13-75; in Karnataka, AHI was 20-70 and BI 40-200; and in Maharashtra, AHI was 10-30 and BI 30-50.

CHIK isolates obtained in C6/36 cell culture during current investigations and viruses isolated during different epidemics in India (1963-2000) were analysed . For initial screening we used alphavirus genus-specific primers (NS4). Phylogenetic analysis based on this region gave discrepant results, the most significant being placement of the mosquito isolate (2000) in Central African genotype as against Asian genotype reported earlier. As a result, we

resequenced all the strains in the E1 gene recommended for phylogenetic analysis. Phylogenetic analyses based on E1 and NS4 regions yielded identical results. The Indian viruses isolated during 1963-1973 belonged to Asian genotype whereas all the current isolates representing three states and the 2000 isolate from the state of Maharashtra grouped with Central/East African genotype. Within Asian genotype, all the older isolates (Indian isolates, 1963-71 and Thai isolates, 1962-78) clustered together whereas later isolates from Philippine (1985), Indonesia (1985), Thailand (1988, 92 & 96) and Malaysia (1998) formed a distinct cluster. The sequence from Reunion Islands representing recent outbreak of the disease also clubbed with the recent Indian isolates. Percent nucleotide identity (PNI) within earlier (1963-73) and recent (2005-2006) Indian isolates was 99.71 + 0.16 and 99.94 + 0.05% respectively whereas PNI between these isolates was 96.11 + 1.09%. The 2005-06 Indian isolates were 98.61 + 0.6% and 98.95 + 0.57% identical with the Reunion and Yawat (India, 2000, Mosquito) isolates respectively.

This initial study confirmed Chikungunya virus as the etiologic agent for massive outbreaks of fever cases with arthralgia/arthritis in three Indian states covering a population of 122.5 million. Thus, re-emergence of Chikungunya was shown in outbreak form after a gap of 32 years. All Indian isolates belonged to Central/East African genotype. This was in contrast to the isolates reported till 1973, all belonging to Asian genotype. Sequence from ReUnion Islands (2005-06) and Democratic Republic of Congo also belong to the Central/East African genotype, Reunion island isolate being closer to Indian isolate. Thus, the current epidemic is caused by Central/East African genotype of CHIK virus. Interestingly, an isolate from mosquito from Yawat, a town in the state of Maharashtra in 2000, also grouped with Central/East African genotype. Earlier outbreaks in India were mainly restricted to large cities. In contrast, the current outbreak is predominantly rural in nature. Isolation of several strains of CHIK virus from adult Ae aegypti signifies importance of this species as main vector in India. Immediate attention on vector control measures was stressed.

Full Genome Sequencing

We determined full genome sequences of CHIK isolates, one each, from five affected states during the 2005-06 episode, one isolate from a mosquito in 2000 (Yawat, Maharashtra state) and two strains isolated during the epidemic in 1963 and 1973. An attempt was made to determine the association, if any, of mutations in the genome with the increased transmissibility of the virus leading to a large epidemic affecting 13 states within one year. Eight CHIK viruses were also sequenced during the present study . These included two viruses of the Asian genotype isolated in 1963 from Calcutta, Eastern India (IND-63-WB1) and in

1973 from Barsi, Western India (IND-73-MH5) along with an isolate each of the 2006 epidemic from 5 states in India i. e., 3 from the states affected earlier, IND-06-KA15, IND-06-AP3 and IND-06-MH2) and two from the states affected 3 months later, IND-06-TN1 and IND-06-RJ1. One isolate recovered from a mosquito in 2000 from western India (ECSA genotype, IND-00-MH4) was also sequenced. The older isolates were obtained from the virus repository of the institute, reconstituted and used.

The phylogenetic analysis based on the available full genomes and E1 gene sequences (1044 nt.) of CHIK viruses was performed using MEGA version 3.1. Clustalx version 1.83 was used to perform the multiple nucleotide and amino acid sequence alignments. For the construction of phylogenetic trees, the neighbor-joining algorithm and Kimura 2-parameter distance model was utilized. The reliability of the analysis was evaluated by a bootstrap test with 1000 replications
.

Complete nucleotide sequence of the CHIK genomes

Full genomes of all the CHIK isolates were amplified in 8 overlapping fragments. Table 3 provides the lengths of genomic RNA and 3'/5' NTR fragments of all the 8 Indian CHIK isolates as compared to the reference S27 strain (genomic RNA, 11805 nt; 5' NTR, 76 nt; 3'NTR, 526 nt). The sequences from the earlier cases of the outbreak were from the states of Karnataka (11729 nt), AP, (11779 nt) and Maharashtra (11800 nt), while sequences corresponding to the cases occurring 3 months later were from Tamilnadu (11750 nt) and Rajasthan (11767 nt) states. In addition, the earliest isolated strain from the Kolkata-1963 epidemic (11784 nt), the last strain isolated during the 1973, Barsi epidemic (11805 nt) and a strain incidentally isolated from a mosquito in 2000 (Yawat, 11814 nt) were also sequenced. Of these, Kolkata and Barsi isolates represent the Asian genotype while all others belong to the ECSA genotype

Phylogenetic analyses

Figure 1a depicts the phylogenetic tree based on full genome analysis. All the Indian isolates from the 2005-06 resurgence representing the five affected states, the isolate from Yawat in 2000, all Reunion isolates (2005-06) and the S27 and Ross isolates (1952) clustered together into the ECSA genotype. The earlier Indian isolates (1963 and 1973) belonged to the Asian genotype whereas the Senegal strain formed a distinct branch (West African genotype). Similar results were obtained when the structural and non-structural regions were analysed separately (data not shown).

As the E1 gene sequences were available for several additional isolates, a separate phylogenetic tree was constructed for the same (Figure 1b). This analysis brought out that all the isolates from the 05-06 outbreaks in Reunion

islands and India clubbed together whereas the Yawat-2000 and Uganda-1982 isolates sharing 99% nucleotide identity constituted a separate branch. Similarly, the isolates from the Democratic Republic of Congo (2000) formed a separate cluster within the same genotype whereas the 1996 isolate from the Central African Republic remained as a separate branch. Isolates from Thailand recovered in 1975 and 1996 and the 1965 Indian and 1985 Indonesian isolates

Figure 1a: Phylogenetic trees depicting genotypic status of the Indian CHIKV isolates on the basis of (a) full-length genome sequence of 17 isolates

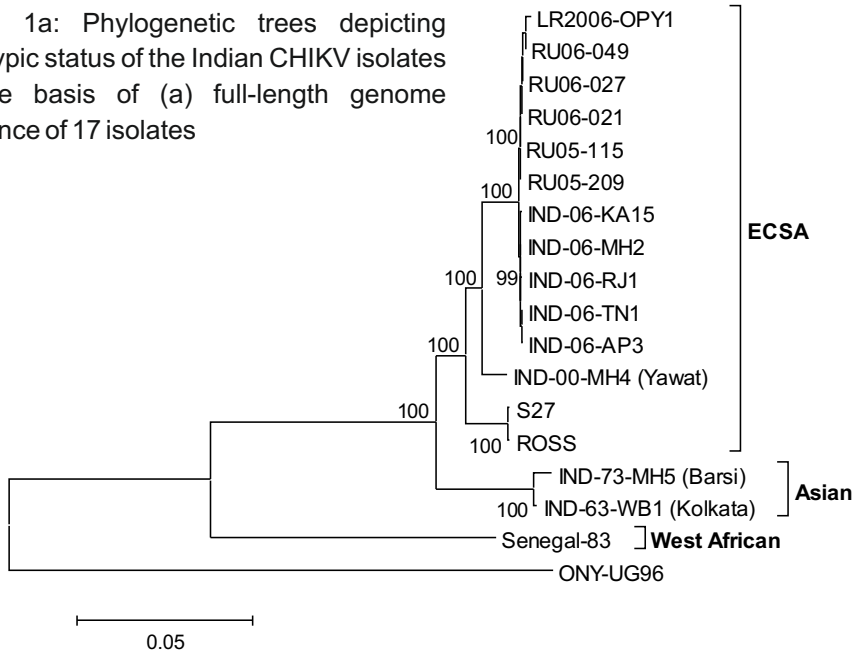
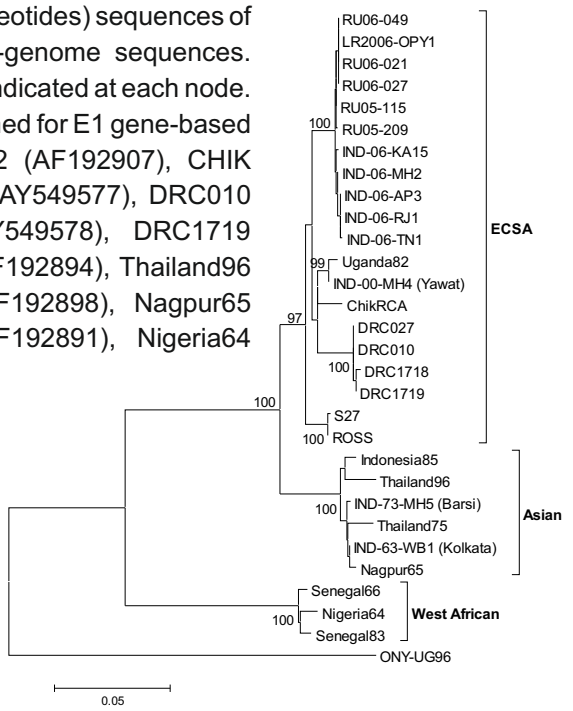


Figure 1b: E1 gene (1044 nucleotides) sequences of 29 isolates including 17 full-genome sequences. Percent bootstrap support is indicated at each node. The additional isolates examined for E1 gene-based analysis included: Uganda82 (AF192907), CHIK RCA (AY549583), DRC027 (AY549577), DRC010 (AY549576), DRC1718 (AY549578), DRC1719 (AY549579), Indonesia85 (AF192894), Thailand96 (AF192900), Thailand75 (AF192898), Nagpur65 (AY424803), Senegal66 (AF192891), Nigeria64 (AF192893)



Sequence Comparisons

Irrespective of the place of isolation (Reunion islands or India), the 05-06 isolates were very closely related (99.9% identity). These isolates differed from the S27 and the Yawat-2000 by 2.7% and 1.7-1.8% respectively. The Asian genotype differed from the ECSA and West African genotypes by 4.4 - 5.3% and 15.4 - 15.5% respectively. The African genotypes (ECSA versus West African) were 14.5 & 14.8% divergent. The amino acid identities across the three genotypes varied from 95.2-99.8% .

Sequence analysis of ECSA genotype

Non-structural region

In this region, the recent Indian and Reunion strains exhibited 99.85 - 0.06% identitiy at the amino acid level. As compared to the S27 prototype, 9 identical substitutions were present in both the groups of isolates: Q488R, S589N, A1328V, Y1550H, T1670I, L1794P, P1804S, T1938A and T2117A. There were 7 substitutions viz. L507R, H909Y, V1508I, V1664A, I1709T, S1795N and Q2363L that were shared between the Yawat-2000, IND-06 and RU isolates. The Yawat-2000 strain also showed 6 unique substitutions; V326M, Q1661P, S1691P, C1768R, V1771A and M1782T. The S1691P substitution in Yawat-2000 was also observed in one of the Reunion isolates, RU05-209.

Two unique substitutions were noted in all the IND-06 isolates, both in the Nsp1 region, T128K and T376M. Except for these substitutions, the isolates from the first three states reporting CHIK cases were identical to the RU isolates. Of the two states reporting CHIK cases later, Tamilnadu exhibited one (T1674M in Nsp3) while the state of Rajasthan exhibited 2 mutations (A101V in Nsp1and T1210M in Nsp2).

Structural region

The IND-06 and RU isolates shared 6 substitutions in the structural region, E2-I536T, T637M, S700T V711A; 6K- V756I and E1-D1093E. Further, the two groups had four substitutions (K63R, I284T, A489T and M1078V), identical to Yawat-2000. The unique mutations in the Yawat-2000 strain were A487V, V643M, I702V (in the E2 region) and V828I andA1186T (in the E1 region). The mutation, I702V (E2), though different from all the IND-06 and RU isolates was similar to IND-63-WB1 and IND-73-MH5 strains belonging to Asian genotype.

Two unique mutations, P23S and V27I, in the capsid region were noted among five and four IND-06 isolates respectively. The V27I substitution was not recorded in the AP isolate. Further, Karnataka displayed one (K1020N, E1) and Tamilnadu two (N80D, capsid and V1022I, E1) mutation(s) respectively.

Sequence analysis of Asian Genotypes

CHIK viruses of Asian genotype isolated in 1963 and 73 after a gap of 10 years showed 99.4 (99.72) and 99.39 (99.44) % nucleotide (amino acid) identities in the non-structural and structural regions respectively. These strains exhibited several amino acid substitutions when compared with the S27 strain.

Non-structural region

There were 8, 6, 17 and 8 unique substitutions in the Nsp1, Nsp2, Nsp3 and Nsp4 regions respectively in the two strains of Asian genotype when compared with S27 strain. In addition, unique amino acid substitutions were recorded for the IND-63-WB1 strain (Nsp1: V451M, Nsp3; V1402A). The IND-73-MH5 strain displayed several unique substitutions as S765P, K1028T (Nsp2), S1571N (Nsp3) and D2427E (Nsp4).

Thirteen mutations were shared between the IND-06, RU, Yawat-2000 and the Asian isolates. These included: L172V, E234K, M383L, I384L (only in IND-73-MH5), T481I, C1177Y, S1178N, P1659S, K1685E, A1715T, I2377T, V2418I and V2467I.

Structural region

Capsid, E3, E2, and E1 regions exhibited 3, 4, 10 and 7 amino acid replacements respectively with reference to the S27 strain. In addition, IND-63-WB1 strain exhibited a single substitution in capsid (K89T) and 6K (A795T) regions whereas the IND-73-MH5 strain showed 4 substitutions: capsid, P32L; E2, I609T; 6K, A795I and E1, I864T. Eleven mutations were shared between the IND-06, RU , Yawat-2000 and the Asian isolates: G382K, I399M, G404E, N485T, L506M, S519G, M592R, S624N, A669T, I802V and V1131A.

Table 3. Viruses sequenced during the present study

Strain	Year	Place of Origin	Passage History	Length (nt) of 5' NTR (3' NTR)	Total sequence length (nt)	GenBank Accession No
IND-06-KA15*	2006	Karnataka	2 in C6/36	45 (447)	11729	EF027135
IND-06-AP3*	2006	Andhra Pradesh	1 in C6/36	57 (485)	11779	EF027134
IND-06-MH2*	2006	Maharashtra	1 in C6/36	62 (501)	11800	EF027136
IND-06-TN1*	2006	Tamilnadu	1 in mosquitoes, 2 in mice	63 (450)	11750	EF027138
IND-06-RJ1*	2006	Rajasthan	2 in C6/36	62 (468)	11767	EF027137
IND-00-MH4** (Yawat-2000)	2000	Yawat, Maharashtra	3 in mosquitoes, 3 in mice	77 (500)	11814	EF027139
IND-63-WB1*	1963	Kolkata, West Bengal	6 in mice, 1 in C6/36	66 (481)	11784	EF027140
IND-73-MH5*	1973	Barsi, Maharashtra	1 in mice, 1 in C6/36	62 (506)	11805	EF027141

* Human ** Mosquito

Table 4: Amino acid substitutions in Indian isolates 2005-2006 with respect to S27 strain

Region	Polypeptide Position	**Protein Position**	S27	RU05-209	LR2006-OPY1	IND-06-AP3	IND-06-KA15	IND-06-MH2	IND-06-RJ1	IND-06-TN1	IND-00-MH4
Nsp1	101	101	**A**						V		
	128	128	**T**			K	K	K	K	K	
	326	326	**V**								M
	376	376	**T**			M	M	M	M	M	
	488	488	**Q**	R	R	R	R	R	R	R	
	507	507	**L**	R	R	R	R	R	R	R	R
Nsp2	589	54	**S**	N	N	N	N	N	N	N	
	909	374	**H**	Y	Y	Y	Y	Y	Y	Y	Y
	1210	675	**T**						M		
	1328	793	**A**	V	V	V	V	V	V	V	
Nsp3	1508	175	**V**	I	I	I	I	I	I	I	I
	1550	217	**Y**	H	H	H	H	H	H	H	
	1661	328	**Q**								P
	1664	331	**V**	A	A	A	A	A	A	A	A
	1670	337	**T**	I	I	I	I	I	I	I	
	1674	341	**T**							M	
	1691	358	**S**	P							P
	1709	376	**I**	T	T	T	T	T	T	T	T
	1768	435	**C**								R
	1771	438	**V**								A
	1782	449	**M**								T
	1794	461	**L**	P	P	P	P	P	P	P	
	1795	462	**S**	N	N	N	N	N	N	N	N
	1804	471	**P**	S	S	S	S	S	S	S	
Nsp4	1938	75	**T**	A	A	A	A	A	A	A	
	2117	254	**T**	A	A	A	A	A	A	A	
	2363	500	**Q**	L	L	L	L	L	L	L	L
Capsid	23	23	**P**			S	S	S	S	S	
	27	27	**V**				I	I	I	I	
	63	63	**K**	R	R	R	R	R	R	R	R
	80	80	**N**							D	
E3	284	23	**I**	T	T	T	T	T	T	T	T
E2	487	162	**A**								V
	489	164	**A**	T	T	T	T	T	T	T	T
	536	211	**I**	T	T	T	T	T	T	T	
	637	312	**T**	M	M	M	M	M	M	M	
	643	318	**V**								M
	700	375	**S**	T	T	T	T	T	T	T	

Table 5: Amino acid substitutions in Asian genotype(Calcutta-63 and Barsi-73) with respect to S27 strain

Region	Polypeptide Position	**Protein Position**	S27	IND-63-WB1	IND-73-MH5	Region	Polypeptide Position	**Protein Position**	S27	IND-63-WB1	IND-73-MH5
Nsp1	3	3	**P**	S	S	Nsp4	1906	43	**A**	L	L
	34	34	**P**	S	S		1948	85	**R**	K	K
	253	253	**K**	T	T		1953	90	**S**	A	A
	451	451	**V**	M			1964	101	**T**	I	I
	454	454	**S**	G	G		2098	235	**Q**	R	R
	473	473	**S**	R	R		2143	280	**E**	D	D
	486	486	**D**	N	N		2229	366	**T**	A	A
	491	491	**R**	Q	Q		2427	564	**D**		E
	507	507	**L**	H	H		2445	582	**V**	A	A
Nsp2	551	16	**P**	L	L	Capsid	32	32	**P**		L
	714	179	**I**	T	T		37	37	**Q**	K	K
	753	218	**T**	S	S		78	78	**Q**	R	R
	765	230	**S**		P		81	81	**T**	M	M
	873	338	**K**	M	M		89	89	**K**	T	
	1001	466	**M**	V	V	E3	284	23	**I**	A	A
	1028	493	**K**		T		294	33	**E**	K	K
	1139	604	**A**	V	V		305	44	**R**	S	S
Nsp3	1402	69	**V**	A			321	60	**H**	R	R
	1509	176	**V**	I	I	E2	327	2	**T**	I	I
	1557	224	**T**	I	I		409	84	**F**	L	L
	1571	238	**S**		N		443	118	**S**	G	G
	1616	283	**S**	N	N		474	149	**K**	R	R
	1667	334	**A**	V	V		482	157	**V**	A	A
	1670	337	**T**	A	A		530	205	**G**	D	D
	1682	349	**V**	A	A		571	246	**A**	V	V
	1686	353	**I**	T	T		609	284	**I**		T
	1700	367	**L**	P	P		643	318	**V**	R	R
	1709	376	**I**	V	V		702	377	**I**	V	V
	1714	381	**S**	T	T		709	384	**M**	V	V
	1716	383	**T**	I	I	6K	795	47	**A**	T	I
	1770	437	**V**	A	A	E1	864	55	**I**		T
	1782	449	**M**	I	I		881	72	**N**	S	S
	1791	458	**A**	T	T		907	98	**A**	T	T
	1792	459	**T**	M	M		951	142	**I**	V	V
	1816	483	**N**	D	D		954	145	**T**	S	S
	1850	517	**S**	P	P		1020	211	**K**	E	E
							1034	225	**A**	S	S

5' and 3' NTR

The 5' NTR was highly conserved while the 3' NTR showed maximum divergence (10.1 17.4% between different genotypes). Within 3' NTR, the Asian genotype was characterized by insertion of 10 nt between position 11377 and 11378, 11nt between position 11514 and 11515 and one insertion at 11425 with respect to S27, Similarly, several deletions were reported in the Asian genotype as compared to S27. These included two deletions at position 11465-11466, one deletion at 11595 and another deletion at 11629. The IND-73-MH5 strain exhibited unique deletions at 11436, 11743 and 11744 when compared to the S27 strain. A stretch of nineteen A nucleotides, a possible Internal poly-A site in S27 showed six substitutions in the Asian genotype (figure 2). The deletion of a stretch of fourteen of the nineteen A nucleotides reported for the RU isolates was maintained in all the Indian isolates belonging to ECSA genotype. In addition, the AP strain showed one insertion between 11579 and 11580 and two deletions at positions 11629 and 11800.

Overall, the full genome analysis documented that isolates causing enormous number of cases in Reunion and India are caused by the same strain and not the result of recombination of Asian and ECSA genotypes (Given below).

```
S27          CTAATAATC- ---------T GTAGATCAAA GGGCTATATA ACCCCTGAAT  [140]
RU05-209     .A.......- ---------. A......... ......CGC. ..........  [140]
LR2006-OPY1  .A.......- ---------. A......... ......CGC. ..........  [140]
IND-06-AP3   .A.......- ---------. A......... ......CGC. ..........  [140]
IND-00-MH4   .........- ---------. A......... ......C.C. ..........  [140]
IND-63-WB1   ....G....A ATAGATAAG. A......... .....GA.C. ..........  [140]
IND-73-MH5   ....G.C..A ATAGATAAG. A......... .....GA.C. ..........  [140]

S27          AGTAACAAAA TACAAAA-TC ACTAAAAATT ATAAAAAAAA AAAAAAAAAA  [190]
RU05-209     .......... .......-.. .......... .......--- ----------  [190]
LR2006-OPY1  .......... .......-.. .......... .......--- ----------  [190]
IND-06-AP3   .......... .......-.. .......... .......--- ----------  [190]
IND-00-MH4   .......... .......-.. .......... .......--- ----------  [190]
IND-63-WB1   .......... ..T....A.. .A......A. CAT....T.G ....CC.G..  [190]
IND-73-MH5   .......... ..T....A.. .A......-. CAT....T.G ....CC.G..  [190]

S27          ACAGAAAAAT ATATAAATAG GTATACGTGT CCCCTAAGAG ACACATTGTA  [240]
RU05-209     -......... .C........ .......... .......... ..........  [240]
LR2006-OPY1  -......... .C........ .......... .......... ..........  [240]
IND-06-AP3   -......... .C........ .......... .......... ..........  [240]
IND-00-MH4   -......... .C........ .......... .......... ..........  [240]
IND-63-WB1   ......G--. .GG...GA.. .....T.... .......... .....CCA..  [240]
IND-73-MH5   ......G--. .GG...GA.. .....T.... .......... .....CCA..  [240]

S27          TGTAGGT--- --------GA  [260]
RU05-209     .......--- --------..  [260]
LR2006-OPY1  .......--- --------..  [260]
IND-06-AP3   .....T.--- --------..  [260]
IND-00-MH4   .......--- --------..  [260]
IND-63-WB1   .A...C.AAG AATCAATA..  [260]
IND-73-MH5   .A...C.AAG AATCAATA..  [260]
```

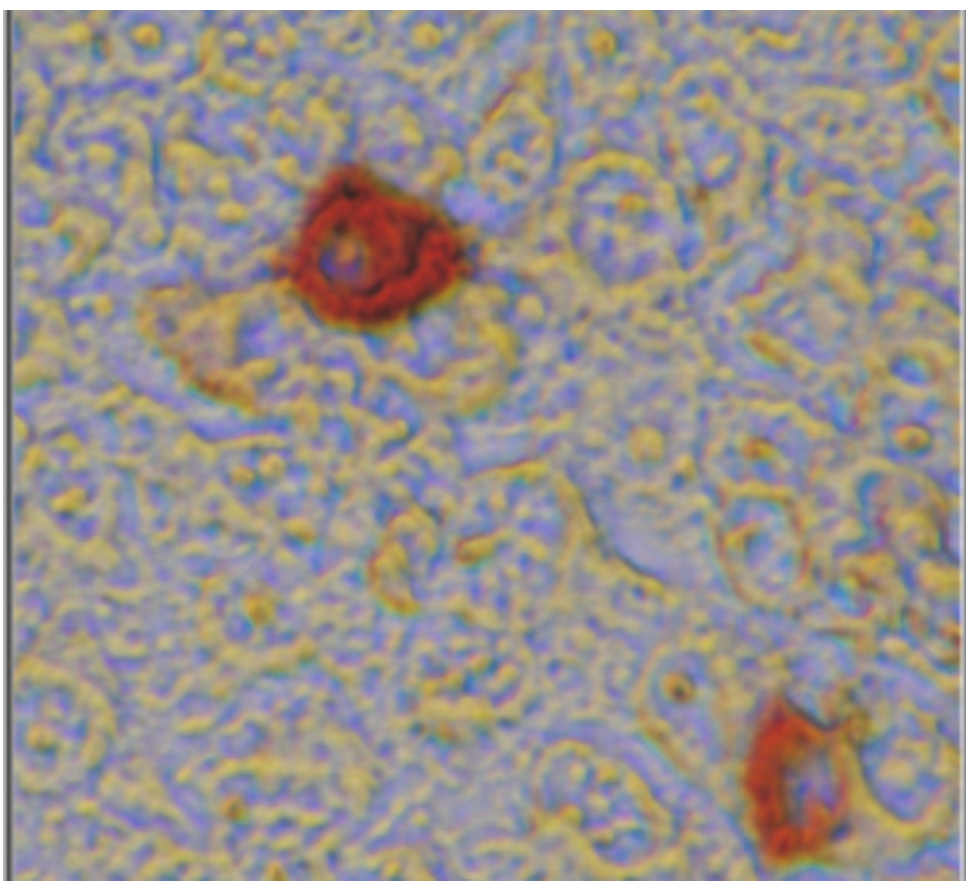
Figure 2 : Alignment of partial 3'NTR sequences of CHIK viruses. The positions do not match the prototype S27 sequence as several strains exhibited deletions/additions. Dots indicate consensus. Dashes indicate the absence of a nucleotide at that position.

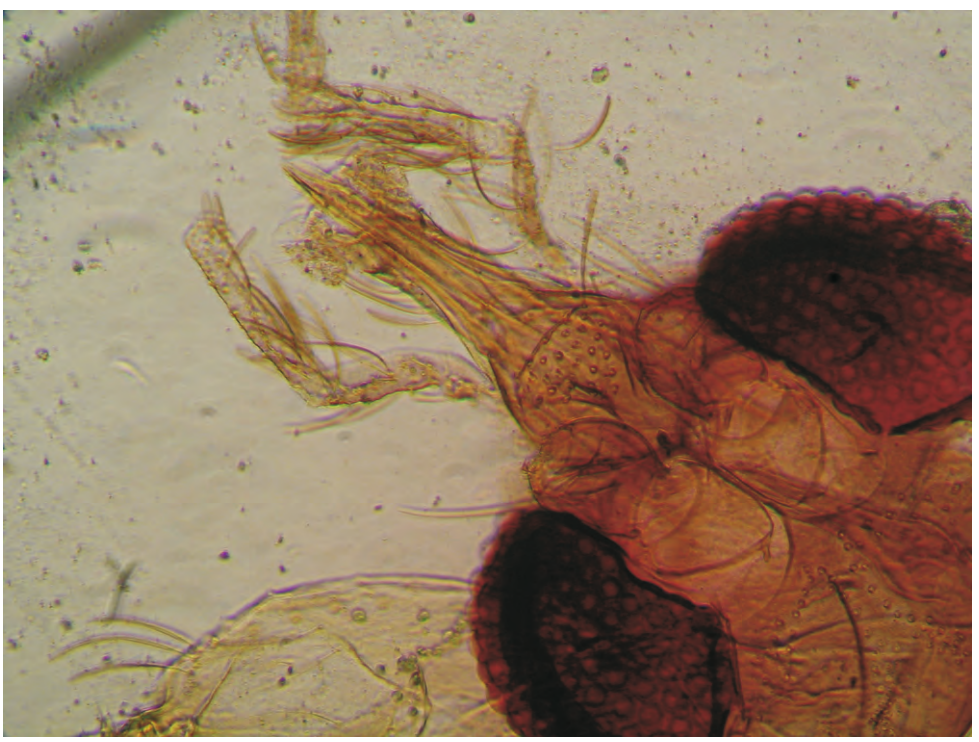
Publications

01. Arankalle VA, Shrivastava S ,Cherian S, Gunjikar RS, Walimbe AM,Jadhav SM, Sudeep AB, Mishra AC.Genetic divergence of Chikungunya Viruses in India(1963-2006)with special reference to the 2005-2006 explosive epidemic. *J Gen Virol* 2007, 88: 1967-76

02. Yergolkar PN, Tandale BV, Arankalle VA et al. Chikungunya outbreaks caused by African genotype, India. *Emerg Inf Dis* 2006: 12; 1580-1583

Chandipura Virus





Probocis of a sandfly

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Analysis of host immune response against Chandipura virus in murine model

Author: B.Anukumar and A.C.Mishra

Passive immunization

In our earlier study we reported that this virus may not induce neutralizing antibody in young susceptible mice. In this study passive immunization was carried out with rabbit anti Chandipura antibody (neutralizing titer 10240) to find out the role of neutralizing antibody in protection. Four groups of 13 day old mice were used in this study (8 mice/group). The first group of mice received only PBS and kept as a control. Second and third group of mice received 50µl of 107 TCID50/ml of virus through intravenous route. In second group of mice the antibody treatment was started one day before the viral infection. The treatment was followed upto three day PI. The animals observed for mortality pattern upto 30days. Mortality was not observed in second group of mice. This experiment concluded that passive immunization completely protected the mice from viral infection.

Virus growth in splenocytes and macrophages

Purified splenocytes and macrophages from 13 and 30 day old mice were infected with 10 MOI of virus. After 24h PI, supernatant from infected cells were titrated in Vero cells. We observed that only 13 day old mice splenocytes and macrophages supported virus replication.

Effect of virus infection on splenocytes

Virus induced apoptosis in splenocytes was studied by Annexin V binding assay. The splenocytes were infected with different concentration of virus (0.1, 1.0, 10, and 20 MOI). At 5h PI, the cells were stained with Annexin V and analyzed by FACS. The result indicated that direct virus infection may not induce apoptosis in splenocytes.

Level of CD4+ , CD8+ and CD19+ cells in splenocytes infected with virus

Adherent cells depleted splenocytes and total splenocytes from both 13 and 30 day old mice were infected with 10MOI of virus. After overnight incubation the cells were stained with anti mouse CD4, CD8 and CD19 antibodies for FACS analysis. We observed that proliferation of CD4+ T cells simultaneously reduction in CD19+ cells in virus infected splenocytes from 13 day old mice. No significance difference in 30 day old mice splenocytes.

Effect of infected RAW cells on normal splenocytes

RAW cells were infected with different MOI of virus and co cultured with normal splenocytes. We found that dose dependent reduction in CD8+ T cells and also reduction of CD4+ T cells. No changes noticed in CD19+ B cells. These results indicated that virus infected macrophages might play a role in apoptosis of lymphocytes.

Cytokine secretion assay

Splenocytes purified from normal mice was infected with virus. At 24h PI infection the cells were tested for secretion of IL-4, IL-10 and IFN-γ by cytokine secretion assay kit. It was observed 9.29% of CD4+ and 11.83% of CD8+ cells were positive for IL-4. Similarly the splenocytes from 48h Post infected young mice 22% of CD4+ and CD8+ cells were positive for IL-10 and 10-13% of CD4 and CD8 cells were positive for IFN-γ. These cells were negative for cytokines when stimulated with specific antigen. This experiment concluded that IL-10 secreted T cell may act as regulatory cells to control the T cell proliferation.

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

Purification of recombinant M protein expressed in E.coli

The recombinant M protein was purified as inclusion body and raised hyper immune serum in guinea pig.

Expression of M gene in eukaryotic system

Cloned M gene in pVAX vector was expressed in VeroE6 cells. The expressed M protein was analyzed by indirect immunoperoxidase test (IIPT). (Fig 1, 2).

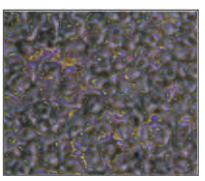


Fig.1. Control

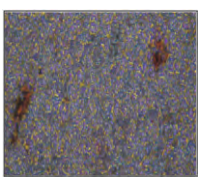


Fig.2. M gene Transfected

Expression of N gene in eukaryotic expression system

Full length N gene was cloned into pFLAG-CMV expression system. The expressed N protein was analyzed by both western blot and IIPT. In IIPT it was found that this protein induced cell lysis. (Fig 3, 4, 5)

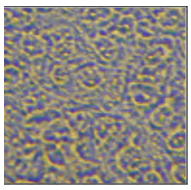


Fig.3. Control

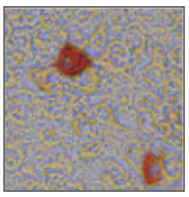


Fig.4 N gene transfected

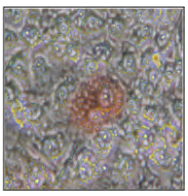


Fig.5. cell lysis by rN protein

TUNEL assay for apoptosis

Recombinant N protein induced cell lysis was confirmed by TUNEL assay. TUNEL positive cells were found in all over the transfected cells (Fig. 6,7,8). This result indicated that expression of N protein induces apoptosis in cells.

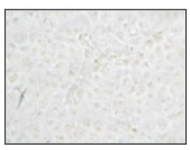


Fig.6 Negative control

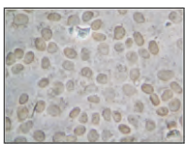


Fig.7 positive control

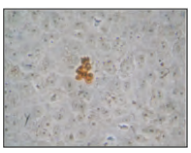


Fig.8.N gene transfected

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

FACS analysis of virus infected RAW cells

Virus infected and LPS treated RAW cells were stained with PE conjugated TLR4/MD2 antibody for expression of TLR4 receptor by FACS. We observed that both virus infected as well as LPS treated cells up regulated the TLR4 receptor expression in RAW cells (Fig. 9).

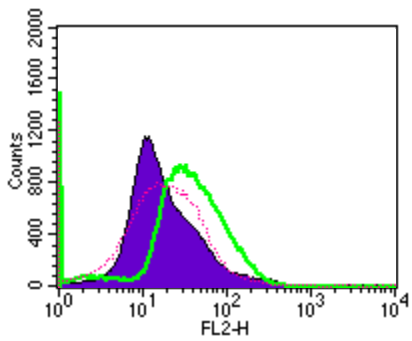


FIG. 9: FACS analysis of virus infected RAW cells

Blue- uninfected RAW cells: Rose- virus infected RAW cells: Green- LPS treated RAW cells

TLR4 receptor blocking assay

Blocking of TLR4 receptor by anti TLR4 antibody did not inhibit the virus entry and replication. So TLR4 might not be a sole receptor for Chandipura virus.

Co- immuno precipitation assay

The virus and TLR4 receptor complex formed in virus infected RAW cell lysate was treated with anti Chandipura antibody. The complex of virus, TLR4 and anti

CHP antibody was pulled down by Protein A sepharose beads. The presence of TLR4 receptor protein was confirmed by western blot using rabbit anti TLR4/MD2 antibody. The end result was visualized by chemiluminescent detection method. The TLR4/MD2 specific bands were observed in western blot. This experiment indicated that the interaction between Chandipura virus and TLR 4 receptor in virus infected RAW cells.

Diagnostic test done in the lab

We have received human sera (191) from various parts of India viz Andra Pradesh, Nagpur, Bangalore and Beed. None of the samples were found positive for Chandipura specific IgM.

We have also received serum samples of frog (33), lizard (14) and Rodents (32) from Entomology group. Serum neutralization test was carried out on these samples. Out of that 2,2,26 samples were positive for NT in frog, lizard and rodents respectively.

Cloning and expression of G-gene of CHPV and evaluation of immunogenicity in mice

The G gene of CHPV (0327 strain from Andhra Pradesh outbreak, 2003) was cloned and expressed in Baculovirus Expression system. A protein of ~60KDa (expected size of G protein) was observed on SDS-PAGE and was confirmed by western blot. The rGp was predominantly present in the supernatant of Sf9 cell cultures, though detected in the cell pellet as well (figure 10). The rGp was concentrated using Amicon gel filtration-based columns. The protein was purified employing HPLC. Preliminary experiment in mice showed that rGp is immunogenic and protects mice following intracerebral challenge of 100LD50 of the homologus virus. For the detection of anti-CHP antibodies rGp-based ELISA was performed as described in the earlier annual report. The mice sera were also tested in in-vitro neutralization test (NT) and showed the reciprocal NT titres ranging from 10-80. ELISA and NT results were comparable. Dose-response experiment is under progress.

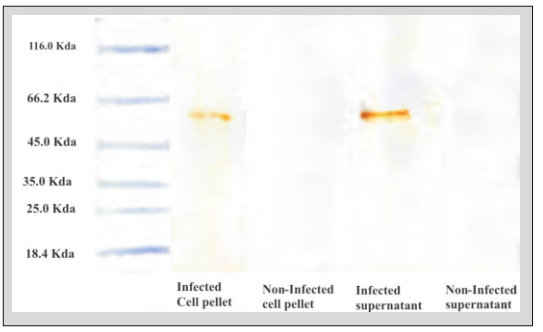


Fig. 10 : Immunoblot of G protein

Evaluation of RNAi (RNA Interference) technology in curing CHP infection in-vitro and in-vivo

An antiviral strategy for CHP is important due to it ability to infect humans. The present study evaluates the possible role of siRNA in controlling CHP infections.

Evaluation of real time RT-PCR using clinical samples

For the evaluation of the real time PCR developed earlier in a clinical setting, serum samples from the following categories were tested (1) encephalitis cases in children [colleted from the Warangal field station, NIV in Andhra Pradesh, during 2005-06 (n=42)] (2) CHIK IgM positive encephalopathy cases (n=8) (3) laboratory confirmed JE cases (n=10) and (4) healthy individuals (n=32). All the samples from the three later categories were scored negative whereas 13/42 samples from Warangal were positive for CHP RNA.

siRNA validation in model system

For evaluating the anti-viral efficiency of G gene and P gene siRNA oligos, we cloned the CHPV G gene (target) upstream in a GFP encoding plasmid pAcGFP1N1. The real time RT- PCR was then used for the quantitative assessment of G gene expression in the Vero cells transfected with both the pSilencer Vector (plasmid encoding siRNA) and pAcGFP1N1 (plasmid encoding G gene of CHPV i.e. target). The siRNA encoding plasmids pSilencer H1G1 and U6G1 showed almost 90% inhibition whereas plasmid pSilencer H1 G3 (with 3 mismatches in the center) exhibited 60% inhibition and U6 G3 was as efficient as the plasmids encoding siRNA with 100% homology with the target (Fig 11).

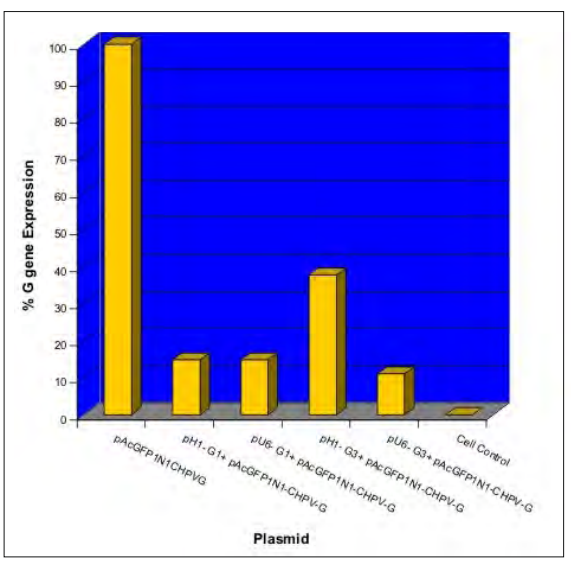


Fig. 11: Efficiency of different siRNA encoding oligos as compared to G gene expression from pAcGFP1N1

siRNA Validation with CHP

Both G and P gene siRNA encoding plasmids alone and in different combinations were used for the transfection of VERO cells with lipofectamine in six-well format. Cells were infected with 360 PFU of CHPV at 18 hour-post-transfection. Cells were observed at 2-hour interval. The figures 12 and 13 show the absence and presence of CPE in the treated cells. At both time points, CPE was observed in controls. There was massive cell death at 24-hour post-infection in treated as well as control cells. A few healthy cells were seen in treated wells. Cells treated with control plasmid, pAcGFP1N1, also showed CPE similar to control. Cells were harvested, RNA isolated and real time RT-PCR was done. Protection as judged by reduction in G gene expression (Fig 14) was observed to vary from 50- 72%. siRNA encoding plasmids gave transient protection to the infected cells. Poor silencing was probably because of lower (~35%) transfection efficiency.

Validation studies with M gene siRNA encoding oligos

M gene siRNA encoding plasmids were cloned into both pSilencer H1 /U6 vectors and were used for the modulating viral replication. M gene siRNA encoding plasmids (pSilencer H1M1 and pSilencer U6H1) reduced the viral gene expression almost by 100%, for low and high viral doses (Fig.12).

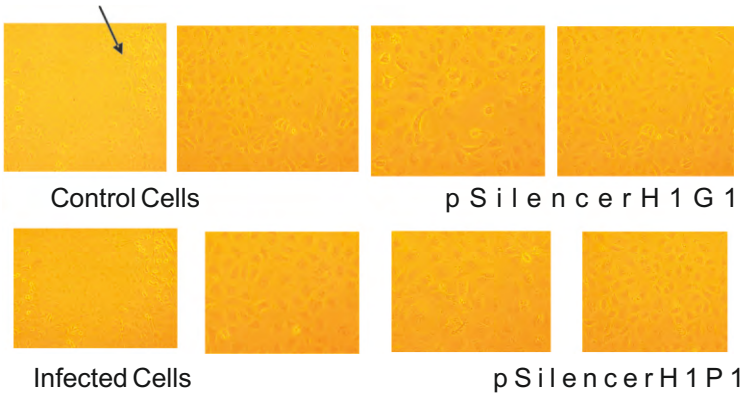


Figure 12 :Vero cells transfected with different plasmids, infected with 360 PFU/ml CHPV. Results are shown at 18 hours post-infection. Control cells infected with the virus showed CPE

Pathogenesis of Chandipura Virus in lab animals

Dr. C.G. Raut

Introduction

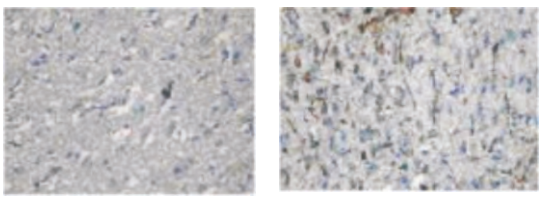
Studies were planned to characterize the pathogenesis of Chandipura virus infection in laboratory rodents by mimicking the natural route of infection and studies were designed to examine the effect of the virus on neuropahrmacologic and histopathologic changes in susceptible animals.

Work Done

Localization of antigen by immunohistochemistry (IHC)

Attempts were made to standardized IHC technique to study the post infection day wise distribution/localization of antigen in different parts of the brain. The results of IHC studies revealed the presence of antigen in cytoplasm of neurons of the brain with varying intensity (Fig. 13 & 14).

Day PI	*IHC Results*
1	++
2	+++
3	+++
4	++
5	+



Negative Positive

Fig. 13: Localization of antigen in cytoplasm of neurons of the brain

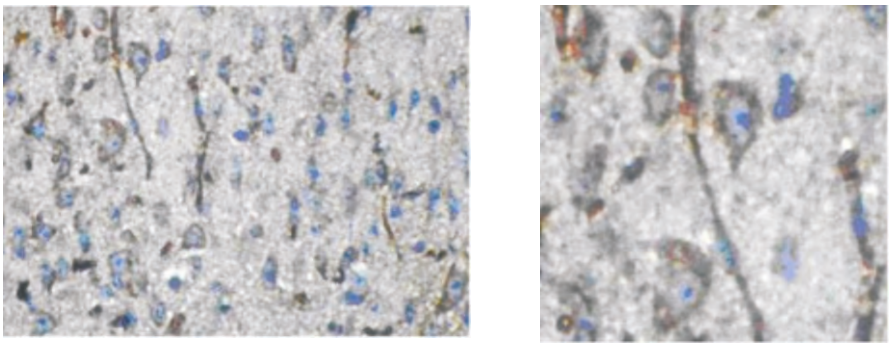
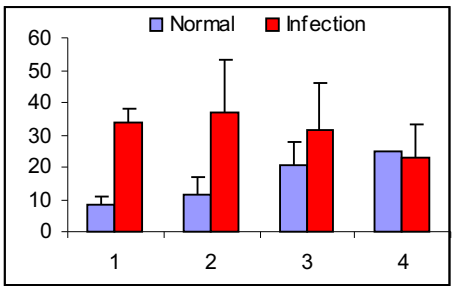


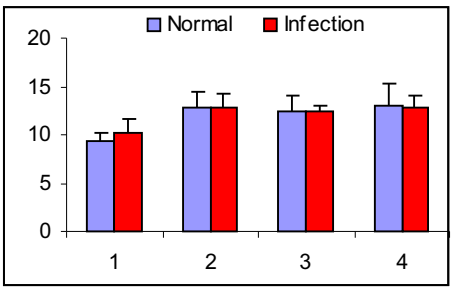
Fig. 14: Positive (brown color staining) (localization of antigen in cytoplasm ofneurons)

Neuropharmacological Studies

Chandipura virus being neurotropic pathogen, attempts were made to study the levels of dopamine and serotonin in the brain & plasma of normal and infected mice. Commercial ELISA kits as well as HPLC techniques were used for detection of serotonin & dopamine. This is being standardized and needs further repetition of the experiment. Serotonin levels from plasma of normal and infected animals were studied at each post infection day. In age group of 24 days, there were increased level observed in infected mice initially as compared to normal. In 10 days old animals there were no significant alterations in the level of serotonin of normal and infected animals.



(Age 24 days)



(Age 10 days)

Publications

1 Arankalle VA, Shrivastava S,Cherian S,Gunjikar RS,Walimbe AM,Jadhav SM, Sudeep AB, Mishra AC. Genetic divergence of Chikungunya Viruses in India(1963-2006)with special reference to the 2005-2006 explosive epidemic. *J Gen Virol* (2007) 88: 1967-76

2 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) 88: 875-884.

3 Mavale MS, Fulmali PV, Geevarghese G, Arankalle VA, Ghodke YS, Kanojia.PC, Mishra AC Veneral transmission of Chandipura Virus by Phlebotomus papatasi (scopoli).*Am J Trop Med Hyg*. (2006) 75 (6)1151-52.

4 Mishra AC. Chandipura Encephalitis: A newly recognized disease of public health importance in India. In: *Emerging Infection* 7. (Chapter 7) edited by WM Scheld, DC Hooper, JM Hughes. (2007) 7: 121-137

5 Mourya DT, Mishra AC. Chikungunya fever. *The Lancet* 2006; 368: 186-187

6 Mavale MS,Fulmali PV, GhodkeYS, Mishra AC, Kanojia P, Geevarghese G. Experimental Transmission of Chandipura Virus by Phlebotomus Argentipes (Diptera: psychodidae). *Am J Trop Med Hyg* (2007) 76 (2) 307-309

Participation in meetings, workshops

Dr A.C. Mishra

Regional Workshop on development of JE vaccine introduction surveillance guidelines organized by WHO from 24 to 26 April 06 at New Delhi

Meeting National Disaster Management Authority, New Delhi with Home Minister at New Delhi, 3rd May 2006

Senior Officials meeting on Avian and Human Influenza from 6 to7June 06 at Vienna, Austria

Symposium on "Zoonotic Diseases" on 22 June 06 at Sahyadri Laboratory, Pune

Meeting of the committee of technical experts to the BSL-3 facility for undertaking the Avian Influenza work at Southern Regional Disease Diagnostic Laboratory on 23 August 06 at Bangalore

Second Annual Review meeting on influenza Surveillance on 25 August 06 at ICMR HQrs, New Delhi

Indian Virology Society meeting on 29 August 06 held at ICAR, New Delhi

JE/DEN/WN meeting on 30 August 06 at Ministry of Health & FW, New Delhi

Second Medical Development Congress on 8-9 September 06 at ICMR HQrs, New Delhi

Meeting regarding status of Avian influenza Research in India : Past, Present & Future on 9 September 06 at DBT, New Delhi

Meeting on Chikungunya Virus Research Programme on 19 September 06 at DBT, New Delhi

Committee to assess the source of infection in outbreak of avian influenza in Maharashtra meeting held on 20 September 06 at ICMR HQrs., New Delhi

Meeting of the Institutional Bio Safety Committee on 23 September 06 at Serum Institute of India, Pune

First National Medical Students' Conference on 5-8 October 06, Pune

Inauguration of Intl Sym on Emerging Trends in Genomic and Proteomic Sciences and felicitation to Prof. NK Ganguly, DG, ICMR from 15-18 October 06 at NIRRH, Mumbai

National Conf. of Indian Association of Microbiologists (India) from 27-30 October 06 at Govt. Med. College, Nagpur

Indo-US Symposium 11-12 November 06, New Delhi

Advisory Committee meeting on Variola Virus Research 16-17 November 06 at Geneva

Discussion meeting on Setting up of a National Agenda for Biosafety from 23-24 November 06 at NIAS, Bangalore

Meeting to decide and recommend issues related to vaccination of poultry against Avian influenza on 20 December 06 at Krishi Bhawan, New Delhi

ICMR Director's Conference from 23-24 December 06 at Jaisalmer

Indo-French (INSERM) meeting on 12 January 07 at ICMR HQrs., New Delhi

Meeting to discuss Inter country programme for preparedness, control and containment of avian influenza under the World Bank funded IDSP project of NICD on 18.1.07 at ICMR HQrs., New Delhi

Meeting regarding 'A prospective open label, uncontrolled single center study for the evaluation of viraemia in health adults after single dose vaccination of JE SA-14-14-2 live alternated vaccine' on 22 January 07 at ICMR HQrs., New Delhi

International Conference on Emerging Trends in Haematology & Immunohaematology from 31 January - 3 February 07at NIRRH, Mumbai

Executive Committee meeting of Indian Virological Society at National Bureau of Plant Genetics Resources on 5 February 07 at New Delhi

Expert Committee meeting on Avian Influenza & Hepatitis Vaccine Project meeting on 6 February 07 at DBT, New Delhi

ICMR CDC Influenza meeting on 6 March 07 at ICMR HQrs., New Delhi

Eighth Sir Dorabji Tata Symposium on Arthropod Borne Vial Infections from 10-11 March 07 at IISc., Bangalore

High Level meeting on responsible practices for Sharing avian influenza viruses and resulting benefits from 27-28 March 07 at Jakarta, Indonesia

Arboviral Diagnostics

A Realtime PCR System

Arboviral Diagnostics Group

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Supply of diagnostic kits and Sero-diagnosis for Flavivirus infections

Padmakar S. Sathe

Introduction

The department of Arbovirus Diagnostics and Virus Registry is one of the service departments and has following major responsibilities

- Provide serological diagnosis for flavivirus infections.
- 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.

Chikungunya virus - During the year ≥20,000 samples were received from ten states and union territories.

The samples collected from patients with febrile arthralgia. were tested for presence of chikungunya virusspecific IgM antibodies using kits developed in-house. The results are presented in Table 1

Table 1: Chikungunya activity in different states

State	Total Districts affected	Samples Tested	CHIK IgM $+^{ve}$
Maharashtra	32	4465	1697
Karnataka	20	767	247
Tamil Nadu	25	1077	469
Andhra Pradesh	21	665	277
Gujarat	12	459	167
Rajasthan	3	99	28
Madhya Pradesh	4	87	42
Orissa	1	34	6
Uttar Pradesh	2	33	26
Kerala	10	77	41
Union Territories	4	114	11
Total	-	7877	3011

Complications of Chikungunya virus infections

Neurological complications: Neurological complications were observed in patients admitted to various hospitals in Maharashtra especially Pune. Most of the patients were admitted with altered sensorium. Serum/CSF collected from these patients were tested for presence of chikungunya virus specific IgM. Sixty-seven serum/CSF pairs (38%) had detectable levels of virus specific IgM. confirming Chikungunya etiology in neurological complications.

Ophthalmic complications: Samples collected from patients with ophthalmic complications after an episode of chikungunya were received from Arvind Eye Hospital Madurai and Thirunelvelli. The complications were mainly optic neuritis and uveitis. There was a positive correlation between chikungunya attack and the ocular complications.

Specificity of Chikungunya MAC ELISA kit

Since Chikungunya emerged as an outbreak almost after three decades, there was no commercial kit for laboratory diagnosis of disease. In-house kit was therefore evaluated for specificity. One hundred and eighty six samples collected from patients with febrile arthralgia / myalgia were tested for presence of dengue and chikungunya virus specific IgM antibodies using dengue and chikungunya in-house kits. Results are presented in Table No 2

Table 2: Specificity of in-house dengue/Chikungunya Kits

Sr. No	Name of place	Total samples	CHIK IgM	Dengue IgM	Positive for both
1	**Mumbai**	**59**	**14**	**8**	**Nil**
2	**Dhule**	**86**	**10**	**7**	**Nil**
3	**Kolhapur**	**13**	**3**	**4**	**1**
4	**Bhilai**	**28**	**9**	**1**	**Nil**
	Total	**186**	**36**	**20**	**1**

Dengue virus serology: Eight Hundred and seventy eight samples (Maharashtra = 612), were tested for dengue specific IgM. Ninety-nine (Maharashtra = 61), samples showed presence of dengue virus specific IgM. Major dengue activity was found in Thane (9/36), Pune (8/27), Sangali (8/40), Delhi (17/56), Badwani, M.P. (9/56).

Japanese Encephalitis Virus serology : Twenty-four samples showed detectable levels of JE specific IgM out of three hundred and twenty three samples tested. Positive samples were mainly from Dibrugarh (11/15).

Supply MAC ELISA diagnostic kits and flavivirus antigens

A total of 154 MAC ELISA Kits were supplied to different organizations.

Twenty-one ml of mouse brain antigen was also supplied during the year.

Table 3: Supply of kits and antigen

Antigen	JE	DEN	CHIK	WN	Total
MAC ELISA Kit	54	46	54	-	154
Antigen (ml)	6	1	8	6	21

Improvements in the existing MAC ELISA kits

Existing kit required two and half days for completion of the test. The procedure has been shortened to Three and Half hours without compromising specificity and sensitivity. The mouse brain antigen has been replaced with cell culture grown antigen for JEV and Chikungunya MAC ELISA Kit.

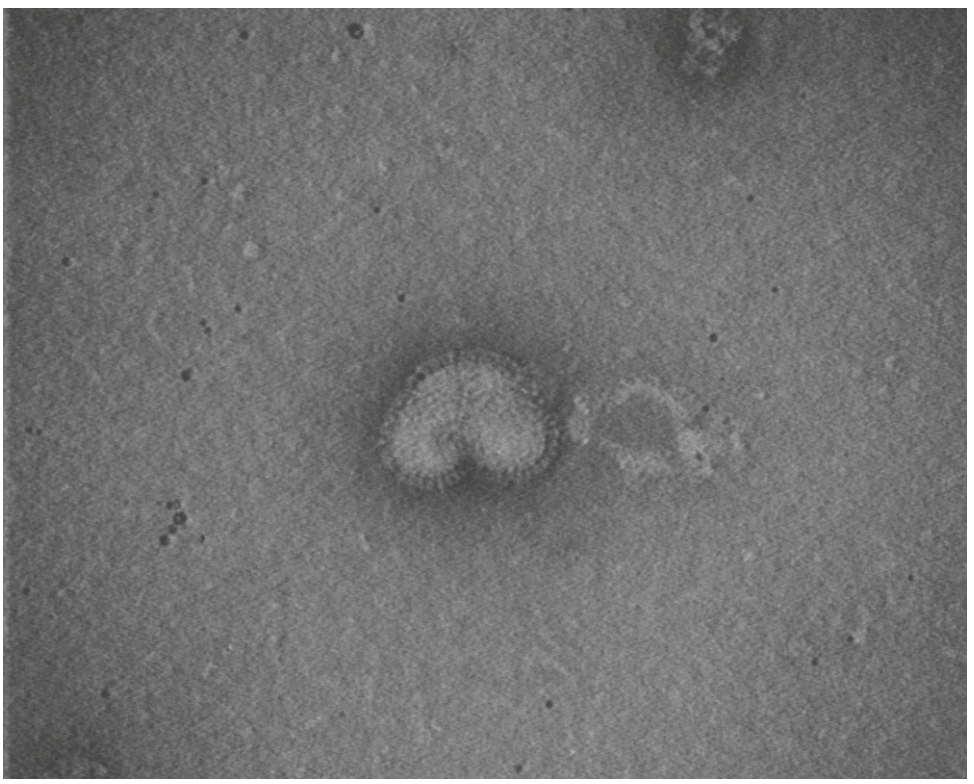
Specimen Registration & supply of virus strains

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 thirty two thousand six hundred and fifty six (32656) samples were registered and issued to different laboratories for diagnosis.

Supply of virus strains. Sixty-seven ampoules of different virus strains were supplied to various research organizations and to different departments within the institute. The virus strains mainly belonged to JE, Dengue 1, 2, 3 and 4 serotypes, WN, Chikungunya and Coxsackie virus strains.

A state of art NASBA platform as a part of the advanced diagnostics infrastructure

Influenza Virus




NATIONAL
INFLUENZA
CENTER


DIFFERENTIAL PRESSURE - AREA WISE
MAIN AIR LOCK
CORRIDOR
CABIN - 1
CABIN - 2
CABIN - 3
TISSUE CULTURE
SAMPLE COLLECTION
MAIN LABORATORY

Influenza Virus Group

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Project Staff

Ms. Shamal R. Waregaonkar

Epidemiological and virological monitoring of human influenza viruses in India

Introduction

This project is an ongoing ICMR-CDC program that focuses on Influenza surveillance serves both as a Regional and Reference Center for influenza. Antigenic and genetic analyses of influenza isolates is carried out and compilation of national data archived.

Activity of the Regional center

Five regional centers have been established in India. Each regional center is responsible for identification of patients with acute respiratory infection and collection of clinical samples from these cases. Further, virus isolation and identification of circulating strains of influenza is carried out. NIV is responsible for influenza surveillance in and around Pune. During the year of reporting, surveillance activity for influenza was continued. 438 patients with acute upper/lower respiratory infections conforming to the case definition were identified from 2 hospitals and 7 OPD/dispensaries. Clinical specimens (throat/nasal swabs and/or nasal pharyngeal aspirate) were collected from them. In addition to the above 145 samples were received from pediatricians and general practitioners from Mumbai. All the above samples were processed for virus isolation in MDCK cell line. Twenty samples yielded influenza isolates, 6 of these were identified as A(H1N1), 4 as A(H3N2) and 10 as type B (antigenically 9 were B/Victoria and 1 was B/Yamagata).

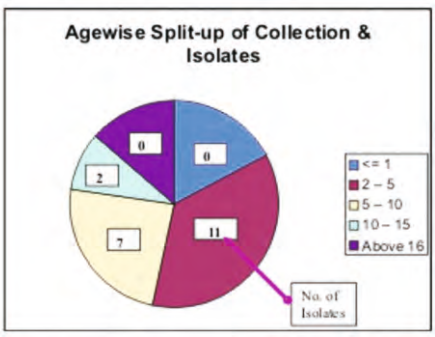


Fig. 1

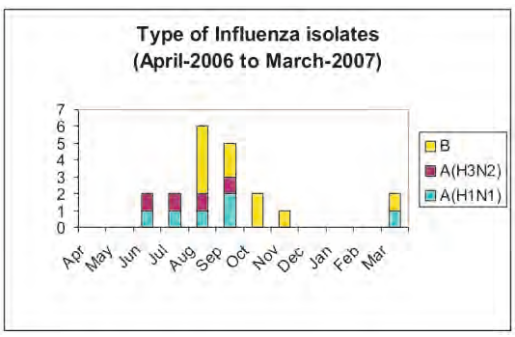


Fig. 2

Activity of the ICMR Referral center

Under the multi-site monitoring project all the five regional centers sent virus isolates to the ICMR Referral center at NIV. This center is responsible for reconfirming the antigenic analysis of the strains received, conduct genetic analysis, dispatch representative isolates to the WHO Collaborating center at CDC, Atlanta and compile national data. NIV also provides technical support to the regional centers whenever required.

Antigenic confirmation of isolates received from regional centers

Delhi: Seventeen virus isolates were received from AIIMS, New Delhi. 14 virus isolates were reconfirmed, 7 as influenza A(H1N1), 6 as A(H3N2) and one type B similar to B/HongKong/330/01.Of the remaining three isolates, 2 isolates did not grow to a sufficient titer and one isolate showed bacterial contamination.

Chennai: Twenty-seven virus isolates were received from KIPM, Chennai. 14 virus isolates were reconfirmed as Influenza A(H1N1) and one type B similar to B/Malaysia/2506/2004 . Results for 6 isolates were discrepant; 3 isolates identified as A(H3N2) at Chennai lab were identified as type B similar to B/Shanghai/361/2002 at NIV, the remaining 3 isolates identified as A(H1N1) at Chennai regional center were identified as A(H3N2) at NIV. Further, 6 isolates could not be grown to a sufficient HA titer and hence were subjected to molecular analysis.

Dibrugarh: 6 isolates along with corresponding clinical samples were received. All the six isolates were identified as A(H1N1) at RMRC, Dibrugarh. Attempts to re-grow these isolates were made at the referral center, but no conclusive results were obtained. However, all 6 corresponding clinical specimens were positive for isolation, and were identified as Influenza A (H3N2).

A second lot of 5 isolates were received for confirmation, due to contamination of the vials received; isolation was not possible, further analysis was carried out by PCR.

Kolkata: 8 isolates were received for identification, virus could not be isolated.

Antigenic characterization of clinical samples received from regional centers

Dibrugarh: 74 clinical samples (nasal swabs) were received from RMRC Dibrugarh, isolation was attempted in MDCK cell line, virus isolate could not be obtained.

Another lot of 100 clinical samples were received from the Dibrugarh regional center, isolation attempts in MDCK cell line yielded 6 isolates of influenza type B which was antigenically similar to B/Malaysia/2506/2004.

Kolkata: 32 clinical samples (nasal/throat swabs), which showed a low HA titer on

isolation in MDCK cell line at the regional center, were received. These yielded 22 isolates, 8 were influenza A(H1N1) and 14 were type B similar to B/Malayasia/2506/04.

One hundred and two influenza isolates were shipped to the CDC in 2 lots, these included 28 from Delhi, 36 from Chennai, 21 from Pune and 5 & 12 from Kolkatta and Dibrugarh respectively. Results of the first lot of isolates sent to the CDC were concurrent with NIV results.

Antigenic comparison among strains of influenza type A(H1N1) of different regional centers

Influenza type A(H1N1) strains similar to A/New Caledonia/20/99 circulated in the year 2005 in regional centers Pune, Chennai, Delhi and Dibrugarh. Antigenic cross reactivity among the strains of A(H1N1) isolated at different regional centers was studied using HI test.

Polyclonal sera were produced by immunizing two fowls for each strain viz 054093 (Pune), 0511134 Chennai), 0512583 (Delhi), 0615324 (Dibrugarh). Fowl were immunized with 10ml of egg-grown virus intravenously on day one and booster dose on day seven and were bled on day fifteen. Cross reactivity was studied by comparing antibody titres in HI test against homologous and heterologous strains. The results were as follows:

Table 1: Antigenic comparison

Regional center	Antigen used for production of immune sera	Homologous and Heterologous titers in HI test			
		Pune	Chennai	Delhi	Assam
Pune	054093	1:320	1:320	1:160	1:160
		1:320	1:320	1:160	1:320
Chennai	0511134	1:640	1:640	1:640	1:640
		1:640	1:640	1:640	1:640
Delhi	0512583	1:160	1:160	1:160	1:160
		1:320	1:320	1:160	1:320
Assam	0615324	1:320	1:640	1:320	1:320
		1:160	1:160	1:160	1:160

It appears that A(H1N1) strains of regional centers of year 2005 appeared to be antigenically similar in HI test.

Quality control RT-PCR for detection and identification of influenza isolates

Isolates received from four regional centers were re-grown and antigenically characterized at NIV. Isolates that could not be re-grown or when antigenic characterization was discrepant in the referral center; RT PCR using diagnostic primers for A and B was carried out. Further, these were subtyped with specific primers for H1 and H3. Positive PCR products were confirmed by sequencing.

Isolates from Delhi: Of the 17 Influenza virus isolates processed for antigenic characterization, 14 were confirmed. Two of the remaining three isolates (F762 & F1202) did not grow to a sufficient titer and one (F779) showed bacterial contamination. All 3 isolates were confirmed as A (H3) [one of these was identified at Delhi center as A(H1N1)] when tested in RT-PCR using diagnostic primers for A and B and further subtyped with specific primers for H1 and H3. Positive PCR products were confirmed by sequencing.

Isolates from Chennai: Of the 27 Influenza virus isolates received, 21 were positive for isolation when processed for antigenic characterization in MDCK cell line. 6 of the 21 isolates showed discrepancy in identification. These 6 isolates were reconfirmed by PCR and sequencing.

Table 2: Influenza virus isolates

Specimen ID	Identified at Chennai	NIV No.	Identified as Type and Subtype
306	A (H3N2)	0633244	Type B (Yamagata lineage)
431	A (H3N2)	0633246	Type B (Yamagata lineage)
463	A (H3N2)	0634974	Type B (Yamagata lineage)
506	A (H1N1)	0634982	A(H3)
509	A (H1N1)	0634983	A(H3)
518	A (H1N1)	0634985	A(H3)

Six isolates that did not grow to a sufficient titer, were tested in RT-PCR using diagnostic primers for A and B and further subtyped with specific primers for H1 and H3. Positive PCR products were confirmed by sequencing. 5 isolates were confirmed as Type B and one isolate as A(H3).

Genetic Analysis of Influenza virus isolates from India

Dr VA Potdar, Ms SR Waregaonkar

Influenza virus genome are well known to undergo antigenic drifts that enable escape from preexisting immunity and potentially cause epidemics in humans. Monitoring antigenic and genetic variations in circulating influenza viruses is crucial for anticipating epidemics and for vaccine design.

Under Multi-site monitoring project Influenza group NIV Pune the referral center carries out genetic characterization and compilation of national data for isolates referred to NIV from four regional centers. HA gene is important gene, which comprises for HA1 subunit. Five antigenic sites lie on HA1 gene, due to drift phenomenon HA1 gene is constantly mutating to produce immunologically distinct strains of influenza virus that cause outbreaks.

Phylogenetic analysis of type A (H3N2) influenza viruses

80 isolates collected during Sept 2004 to March 2007 were received from five regional centers. Forty-eight isolates that were collected up to September 2006 were processed for Phylogenetic analysis by sequencing the 987-nucleotide region in the HA1 gene. These included 9 isolates from Pune, 28 from Delhi, 10 from Chennai and one from Kolkata.

Phylogenetic analysis of the HA1 proteins showed two distinct lineages i.e. A/Panama/ 2007/99 and A/Fujian/411/02, which were the 2003-2004, and 2004-2005 vaccine recommended strains respectively. Fujian like lineage appears to be the ancestor for 2 sub clusters comprising of the A/Wisconsin/2005-like and A/California /2004-like sequences. 12 Isolates from Delhi collected during December 04 to February 05 clustered with A/Panama/ 2007/99 (the 2003-2004 vaccine recommended strain). Four isolates collected during January 05 and March 05 and two isolates in January 06 from Delhi, 3 Isolates collected during October 05, March and July 06 from Pune, and one isolate collected in October 06 clustered together with Wisconsin 05 (2006-2007 vaccine recommended strain). 5 isolates collected between September 04 to April 05 from Chennai, 6 isolates collected between March 05 to January 06 from Delhi and 5 isolates collected during July 05 to March 06 from Pune clustered with A/California /2004 (2005-2006 vaccine recommended strain). Two isolates collected in September and October 2005 from Chennai cluster with A/Fujian /411/02 2004-2005 vaccine recommended strain.

Genetic characterization of the 48 A (H3N2) isolates showed that these isolates were similar to the globally prevalent strains and genetically close to the recommended vaccine component. Results obtained by the phylogeny were in concordance with the antigenic characterization of CDC. (Fig 3)

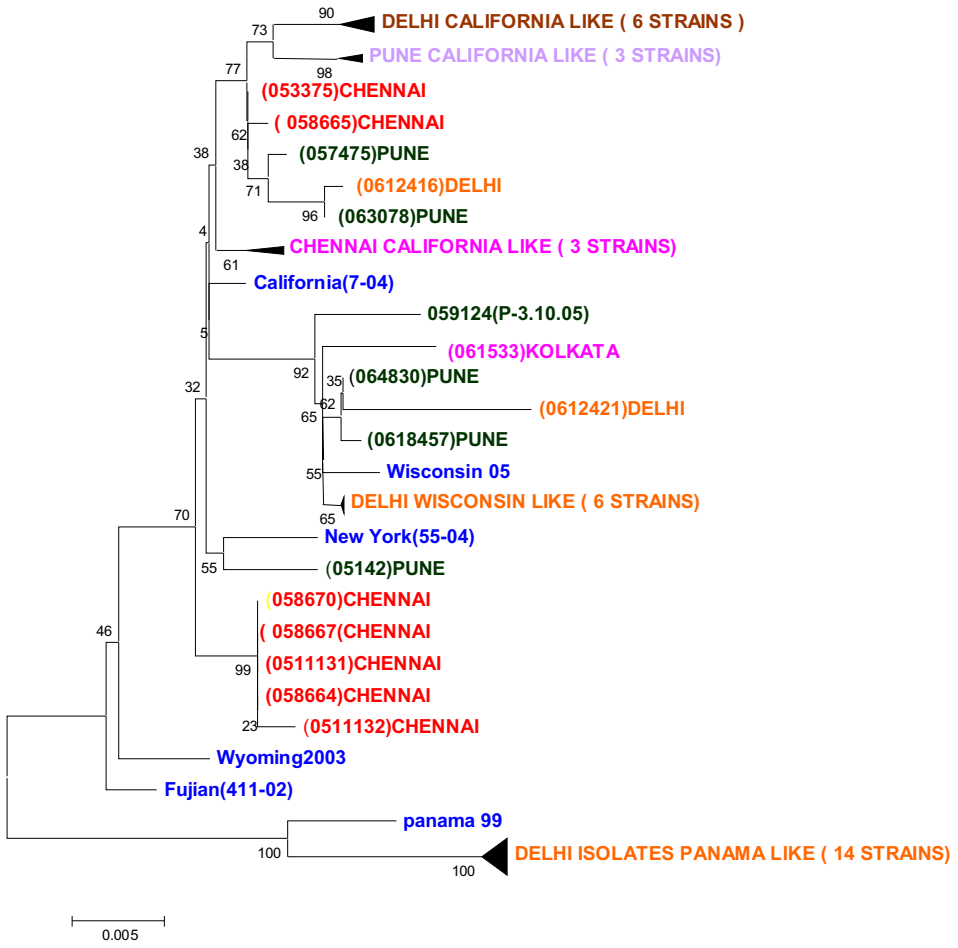


Fig. 3: Phylogenetic analysis of 48 A (H3N2) isolates for HA1 gene .

Phylogenetic analysis of type A(H1N1) influenza viruses

100 isolates were received from five regional centers for the period of September 04 to March 07. Seventeen isolates (Pune- 4, Delhi- 3, Chennai- 6 and Dibrugarh- 4) were processed for genetic analysis by sequencing 987 bp of HA1 gene. Phylogenetic analysis clearly indicated that 17 strains of H1N1 were found to be close to A/New Caledonia/20/99, which is the recommended vaccine strain from 2001 to 2007 for both the hemispheres (Fig 4).

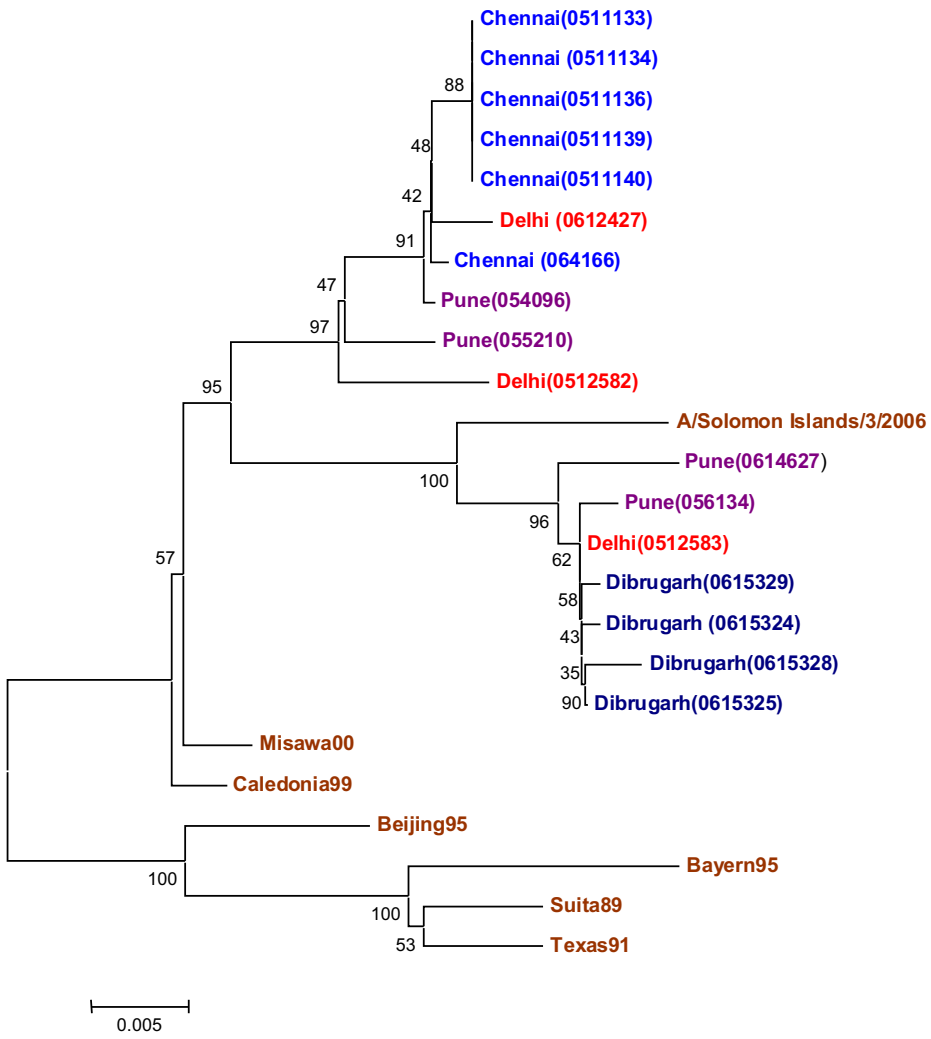


Fig.4: Phylogenetic analysis of 17 A(H1N1) isolates for HA1 gene. .

Phylogenetic analysis of Type B influenza viruses

80 type B isolates collected during September 2004 to March 2007 were received from five regional centers. Out of 80, 29 isolates collected up to March 2006 were processed for molecular analysis by RT-PCR and sequencing. These include 19 isolates from Pune, 4 each from Delhi and Chennai, one each from Dibrugarh and Kolkata. 1406 bp amplicon was generated by type B HA gene specific primers and sequence analysis for HA1 gene of 1044 bp was carried out. The comparison between reference and circulating strains was analyzed by the construction of Phylogenetic trees using MEGA 3.1 version. The neighbor joining algorithm and Kumura 2 parameter distance model were utilized with 1000 bootstrap replicates to evaluate the reliability of the analysis. Phylogenetic analyses clearly indicated that both the lineages of type B were circulating in

India during 04-06. B/Victoria/2/87-like strains were circulating during months of January 05 March 05, strains belonging to both Victoria & Yamagata lineages were co-circulating between August 05-November 05. Again from December 05 to March 06 strains similar to B/Victoria/2/87circulated in India.

WHO recommended B/Shanghai/361/2002 (Yamagata lineage) as type B vaccine component for the period November 04 to April 06. As in India during January 05 March 06 both type B lineages were circulating and viruses of Victoria lineage have predominated, WHO recommended vaccine (04-05 & 05-06) may not have been relevant against Indian strains circulating in 2005-2006 (Fig 5).

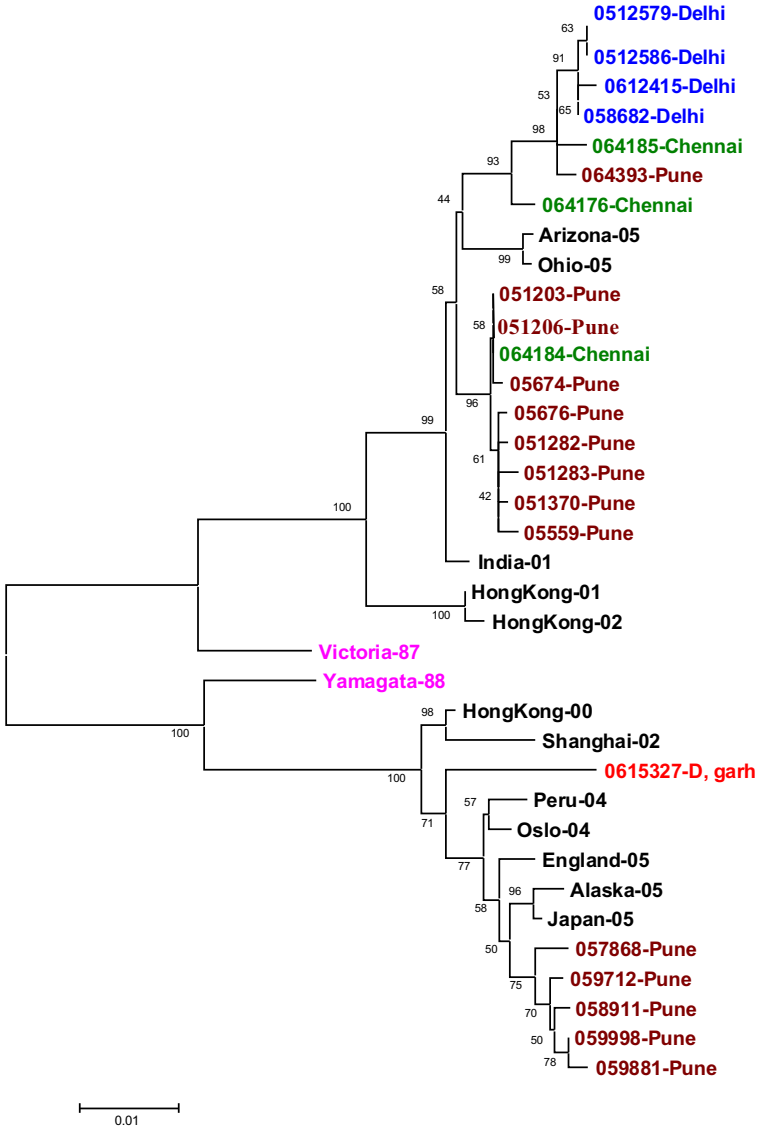


Fig.5: Phylogenetic analysis of 29 Type B isolates for HA1 gene

Detection of amantadine resistance in Pune isolates.

An increase in amantadine resistance in influenza strains has been reported globally. Single and dual point mutation at five known sites resulting in amino acid changes at position 26,27,30,31,or34 in the trans-membrane region of M2 protein are responsible for resistance. This study was carried out to determine amantadine resistance in Pune isolates. A total of 22 influenza A virus strains (12 H1N1 and 10 H3N2) isolated from 2005-2006 were subJEcted to RT-PCR with M2 gene specific primers to obtain an amplicon of 270 bp. The resistant strains grouped together with A/Wisconsin/67/05 with 100% homology. Remaining 6 A(H3N2) strains were sensitive to the drug and grouped with previous vaccine strains according to their percent similarity. The A(H1N1) isolates were sensitive to amantadine and clustered with A/New Caledonia/20/99 with 87.7-100% homology.(Fig 6).

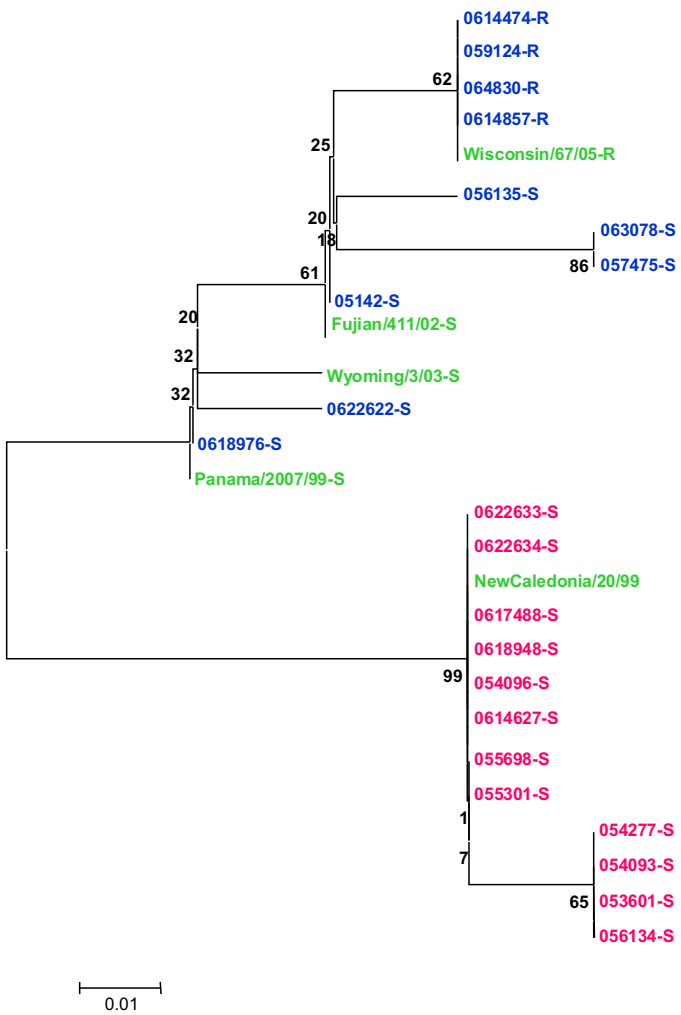


Fig. 6: Phylogenetic analysis of M2 gene based on 65 aa comprising of critical region for amantadine resistance

Influenza pandemic preparedness.

Standardization of one step diagnostic RT- PCR for detection of H5N1

One-step RT PCR for the amplification of HA, NA and M gene of H5N1 was standardized. WHO recommended primers along with earlier reported primers were used. M gene specific primers were also used to amplify the amino acid region responsible for amantadine resistance. To determine the limit of detection of the test, serial RNA dilutions were used for amplification. Results were confirmed by sequencing of PCR products.

Table 3(a): Molecular detection of influenza virus isolates

Gene Target	Primer pair used	Dilution Factor	Expected size in bp	Results
HA	H5-1F, A5H5 (WHO)	Neat	219	+4
		10^{-5}		+3
NA	N1-1, N1-2 (WHO)	Neat	616	+4
		10^{-3}		+2
M	VRY-1, VRY-2 (Potddar et. al.)	Neat	330	-ve
M	ARF1, ARR2 (Pachucki et. al.)	Neat	164	+4

Standardization of One step diagnostic RT- PCR for detection of H9 antigen

Different primer combinations recommended by WHO and primers from other published papers were used to amplify M and HA gene of H9 BPL inactivated WHO reference antigen. The results were confirmed by sequencing of PCR products.

Table 3(b): Molecular detection of influenza virus isolates

Gene Target	Primer pair used	Expected size in bp	Results
M gene	VRY1, VRY2 (Potddar et. al.)	330	+4
M gene	ARF1, ARR2 (Pachucki et. al.)	164	+4
HA gene	HAF1, HAR1 (NIV)	443	+4
HA gene	HAF2, HAR2 (NIV)	357	+4
HA gene	H9-426F, H9-808R (WHO)	403	+4

Standardization of One step diagnostic RT- PCR for detection of H7 antigen

In-house designed primer and primers from published sources were used to amplify M and HA gene of H7 BPL inactivated WHO reference antigen. Results were confirmed by sequencing.

Table 3(c): Molecular detection of influenza virus isolates

Gene Target	Primer pair used	Expected size in bp	Results
M gene	ARF1, ARR2 (Pachucki et. al.)	164	+4
HA gene	H7-2 (Spackman et. al.), H7-4 (NIV)	120	+3

Sensitivity detection of H5N1 Real Time systems

Comparison of three different real time test (NASBA, ABI real time PCR and Rotor gene real time PCR) was done with their respective kits using WHO reference BPL inactivated H5N1 antigen. RNA dilutions from Neat to 10-7 were conducted and alternate dilutions were tested in all the three test systems. All systems detected RNA up to 10-3 dilutions.

Whole genome sequencing of H5N1

Designing and testing of sequencing Primer

An outbreak of avian influenza H5N1was reported in Maharastra in February 2006. In order to sequence all the 8 genes of H5N1 sequencing primers were designed. A total of 67 primers were designed from the conserved region of all the 8 genes.

Gene amplification: cDNA was prepared using Uni 12 primer and RT-PCR was performed using expand Hi-Fi PCR system (Roche) to amplify the PB2, PB1, PA, HA, NP, NA, M and NS genes using Primers and protocol by Hoffman et al. The size of amplified products for PB2, PB1, PA, HA, NP, NA, M and NS were 2341, 2341, 2233,
1778, 1565, 1413, 1027 and 890 bp, respectively, plus 29 mer oligonucleotide primer for all products.

Nucleotide sequencing: Expected size DNA amplified products were purified and sequenced using NIV designed primers.

Studies on other respiratory viruses

Detection of Respiratory syncytial virus and other respiratory viral antigens in respiratory specimens

Damle RG, Chadha MS

142 clinical specimens were collected from pediatric patients attending out patient department (OPD) and admitted (IPD) to Pune Hospital for upper &lower respiratory tract infection. This included 47 nasopharyngeal aspirates (NPA), 73 nasal swab (NS), 17 throat swab (TS), and 5 NS+TS. NPAs were subJEcted to Chemicon IF test for the detection of respiratory viruses and TS/NS specimens were tested in RT-PCR for the detection of HRSV. When tested by IFA, 15 samples were Human respiratory syncytial virus (HRSV) positive, 1 had Para Influenza Virus 3 infection, and another child had concurrent PIV3 and HRSV. Two specimens were positive for VSA i.e. mixture of MAbs to different respiratory viruses, indicating respiratory virus infection.

In a diagnostic semi nested RT-PCR a subset of 92 TS/NS specimens, negative for influenza virus, were tested using conserved F gene primers. This included 68 NS, 11 TS and 13 NPA specimens that could not be tested in IF test due to insufficient cell number. 26 of the 92 samples tested positive for HRSV.

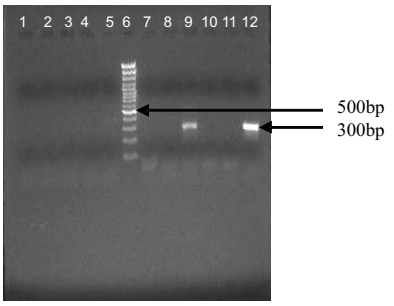
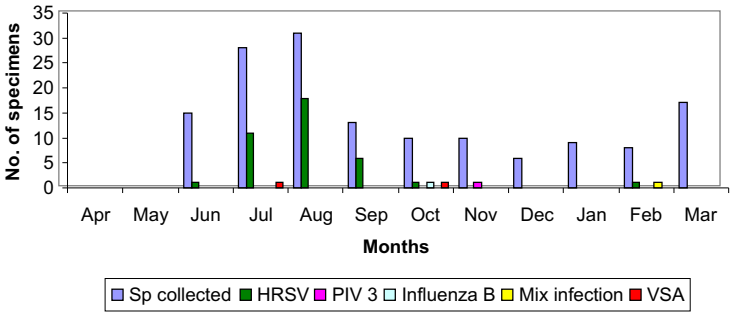


Fig. 7: Lane 1 to 5: First round PCR. Lane 6: 100 bp molecular weight marker. Lane 7 to12: Semi nested PCR Lane 9: Specimen PN06-324. Lane 5 and 12: Positive control. Lane 3 and 8: Negative control

Significant activity of HRSV was noted during monsoon season from July through September as seen in the graph. It is concluded that during 2006-07 HRSV was an important etiological agent as it was detected in 41 (28.87 %) of the cases.

Fig. 8: **Respiratory viruses detected from April 2006-March 2007**



Subgroup analysis and genotyping of human respiratory syncytial virus from clinical specimens

Specimens positive in diagnostic PCR were further subJEcted to genotyping; the 270 nt. hyper variable region of G gene was amplified and sequenced. Genotyping PCR and sequencing were initially tested on tissue culture isolates and found to work satisfactorily, results distinguishing the two subgroups of HRSV viz. group A and B were obtained. Following the satisfactory working of test system on tissue culture, genotyping for clinical specimens was standardized.

Of the 26 specimens positive in diagnostic PCR, 16 grouped as A and 5 belong to group B. Three specimens could not be genotyped because of low RNA count in the samples. Further sequencing and genotyping is in progress.

Retrospective genetic analysis of HRSV strains showed the presence of genotype GA5 in the year 2002. In 2004, circulation of strains of both the groups A and B was observed

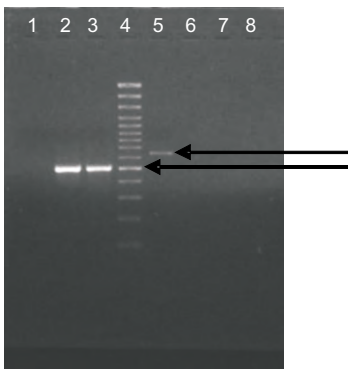


Fig.9: Primer testing using tissue culture isolates
Lane 1-3: with group A specific primers; Lane 1: group B specimens (PN04-4987) negative. Lane 2 and 3: group A specimen (PN02-5513 and PN04-5050) positive. Lane 4: 100 bp molecular weight marker, Lane 5-7: with group B specific primers; Lane 5: group B (PN04-4987) positive. Lane 6 and 7: group A specimen (PN02-5513 and PN04-5050) negative.

Detection of HRSV in antigen capture ELISA and comparison with RT-PCR

A study for detection of HRSV from TS/NS specimens from the year 2004 by NIV ELISA and comparison with RT-PCR was concluded. NIV ELISA is indigenously developed MAb based antigen capture ELISA, where MAbs to conserved phosphoprotein of HRSV are used. In the study, 133 specimens were tested in both the tests of them 12 specimens (10 positive P/N > or = 2.0, 2 borderline positive P/N > or = 1.9) and 121 specimens were negative in NIV ELISA. The 12 ELISA positive specimens were also positive in RT-PCR. However, of the 121 ELISA negative (P/N< 2.0) specimens, 11 specimens were positive and 110 were negative in RT-PCR. Using these data sensitivity, specificity, PPV and NPV of the NIV ELISA was calculated (Table).

Table 4: Comparison of ELISA and PCR for detection of RSV

NIV ELISA	RT-PCR		Total
	Positive	Negative	
Positive	12	0	12
Negative	11	110	121
Total	23	110	133

NIV ELISA had sensitivity 52.17%, specificity 100%, positive predictive value 100% and negative predictive value 89.43%. Lower sensitivity of NIV ELISA can be attributed to the fact that MAbs used in ELISA were raised against subgroup A strain and reacted against conserved P protein of HRSV. In the year 2004 strains of both the groups A and B were circulating but the possibility is that MAbs might have recognized only A strains and failed to detect B strains leading to lower sensitivity of the ELISA.

Avian influenza virus Group

The Avian Influenza division was established in October 2006.

Avian influenza A H5N1virus isolation and molecular characterization

K. Ray, SD Pawar, AK Chakrabarti, ,VA Potdar, Ms. SR Waregaonkar and Ms. AA Joshi, SS Cherian, SM Jadhav, DR Patil.

Introduction

The laboratory diagnosis and molecular identity of avian influenza (AI) viruses can provide important clues to virus origin, evolution and plan vaccine strategies of public health importance. The present project focuses on the molecular characterization of AI virus strains from India.

Summary of work

Post-mortem chicken tissue samples collected during the first poultry outbreak of avian influenza A H5N1in 2006 from Navapur, Nandubar district of Maharashtra, were processed for virus isolation in accordance with WHO recommendations in the enhanced BSL3 facility at the Microbial Containment Complex, NIV. Isolates were obtained from two chickens in both MDCK and SPF chicken egg allantoic fluid. The isolates were identified as influenza A, H5N1 by Haemagglutination (HA), Haemagglutination Inhibition (HAI) assays, rapid test (QuickVue), and by reverse transcription polymerase chain reaction (RT-PCR) with primers specific for the HA (H5) and NA (N1) genes. Eight segments of the two MDCK isolates of H5N1 viruses (A/Ck/India/NIV33487/06 and A/Ck/India/NIV33491/06) were fully sequenced and characterized. All segments in the two isolates were identical except for one difference (nt 740) in the M gene. All the genes belonged to clade 2.2 of the Z genotype (Qinghai like).

The occurrence of closely related viruses in the East Africa/West-Asia flyway of migratory birds suggests that the viruses in India may have been introduced through migratory birds. Molecular markers suggest that the NIV isolates are sensitive to both drugs Oseltamivir and Amantadine. Amino acid residues responsible for pathogenesis, glycosylation and receptor binding were also analyzed. Host specificity markers studied in the HA, M1, M2, NP, PA, PB2 and NS proteins revealed a predominantly avian specificity. The presence of the reported mammalian virulence marker L627 in PB2 and human specific residues, V28 and E92 in M2 and NS1 respectively in the NIV isolates, may be significant There were two specific mutations in the NIV isolates, V247I in NA and A239T in M1, compared to the other clade 2.2 isolates analyzed, whose significance is yet to be ascertained.

Avian influenza (AI) surveillance in migratory, domestic birds in Maharashtra, during avian migratory season 2006-2007.

SD Pawar, SS Koratkar, VV Thite , SS Kode, MB Nanaware, B Pal , S Raut, K. Ray, AK Chakrabarti, AC Mishra .
Field work team: AV Jamgaonkar, SN Randive & S. Pande (ELA foundation, Pune).

The main focus in the last three months (January March, 2007) has been on surveillance for Influenza A H5N1 virus in poultry and water birds. Fecal samples of poultry and water birds were collected from six districts of Maharashtra (Nagpur, Nandurbar, Pune, Raigarh, Satara and Solapur). A total of 1582 samples (each sample consisting of a pool of five similar droppings) were collected till March 31, 2007. These included 398 samples from poultry (383 chicken - from commercial as well as backyard farms, and 15 ostrich samples) and 1184 samples from water birds (ducks, geese, storks, cormorants, gulls etc.). The movement of water birds was monitored at 18 different dam sites in Pune in order to identify dams to be selected as sentinel areas in future. Samples were tested by Reverse Transcription Polymerase Chain Reaction (RT-PCR). One step and two-step RT-PCRs were standardized for the detection of HA (Influenza A universal and H5 specific), NA (N1 specific) and M (Influenza A universal) genes of Influenza A viruses. Sensitivity of the RT-PCRs was at picogram levels. 700 fecal samples were tested with HA and NA specific primers and 200 samples were tested with the M specific primers. All samples were negative for the three genes. The presence of H5N1 was ruled out in these 700 samples. There was a report of a large number of chickens dying in Indapur in the first week of March. 118 poultry samples were collected from the affected areas. All were found negative for avian influenza. 111 samples were passaged in special pathogen free (SPF) embryonated chicken eggs in order to isolate virus. No specific mortality was observed in the first passage. 41 samples were inoculated in the Madin-Darby Canine Kidney (MDCK) cell line. No CPE was observed in the first passage. All isolation protocols were carried out in the BSL-3+ facility.

Reverse Genetics system to generate infectious influenza virus particle

AK Chakrabarti and B Pal and AC Mishra

Reverse Genetics describes the generation of virus possessing a genome derived from cloned cDNA. This de novo synthesis of negative sense RNA viruses from cloned cDNA has revolutionized the research on negative sense RNA viruses like influenza A virus and is a powerful tool for dissecting virus life cycle and study on pathogenesis.

We are working to understand pathogenesis of influenza A H5N1 viruses and to make recombinant vaccine strain against highly pathogenic influenza A H5N1 viruses. A Material Transfer Agreement has been signed with St Jude Children's Research Hospital, Memphis, TN, USA for this purpose. So far all the plasmids has been amplified and tested which will be used to generate infectious influenza virus particle using reverse genetics system.

Publications

01. Arankalle VA, Chobe LP, Chadha MS. Type-IV Indian swine HEV infects rhesus monkeys. *J viral Hepatitis* 2006, 13: 742-745

Participation in meetings, workshops

M S Chadha

Talk during the Workshop on Bio-safety held at the NIV on 1st April at MCC, for junior scientific and technical staff.

Topic: Safe laboratory techniques

12th International Symposium on Viral Hepatitis And Liver Disease, Paris, July 1-5, 2006.

Oral presentation: Outbreaks of hepatitis A in western India: 2002-2004

Meeting at NARI (Jehangir Hospital clinic) to decode a Phase II double-blind placebo controlled trial of "Praneem" Polyherbal tablet for assessing its safety and acceptability as vaginal microbicide. 5th April 2006

Attended: Laboratory Biosafety and Biosecurity Workshop organized by the National Institute of Virology India & Sandia National Laboratories, Albuquerque, US, at Pune, 2- 4 May 2006.

Attended: Workshop on Bioethics in Clinical Research, sponsored by DBT, ICMR & US Department of Health and Human Services (DHHS), at New Delhi, 20-22 June 2006.

Attended session on Influenza at the Asia Pacific Virology Congress, November 15, 2006.

ICMR- CDC Meeting of Influenza Surveillance Network Partners held on 6th March, 2007 at ICMR Hqrs. New Delhi at 2:30PM

Tabletop exercise to critically review contingency plan for Avian Influenza-10-11th January 2007, at National Institute of Communicable Diseases; New Delhi.

Dr . Ray K

Focused meeting on Avian Influenza: "Status of Avian Influenza Research in India: Past, Present and Future". Department of Biotechnology, Ministry of Science and Technology, New Delhi. September 9, 2006.

Dr. SD Pawar, RO

Invited talk in Asia Pacific Conference on Medical Virology on "Influenza surveillance in India", New Delhi, November 13-15, 2006.

Invited talk on avian influza at "Influenza Virus Strain Surveillance" workshop, jointly organized by WHO-CDC-NIV-ICMR at NIV, Pune, January 20-24, 2007.

Participated in the workshop on "Avian Influenza Preparedness and Response" jointly organized by NICD-DGHS-CDC-WHO at New Delhi, March 6-9, 2007.

Trained in Microneutralization Assay for detection of Influenza A H5N1 virus specific antibodies by Dr. Jenna Achenbach from the Influenza Division, Centers for Disease Control, Atlanta, USA, visited the Avian Influenza Division during 25-31 March, 2007.

Completed a Certificate course in ornithology from December 2006 to February 2007, which is jointly conducted by Abasaheb Garware College and ELA foundation (Dr. Satish Pande), Pune.

Worked as a visiting faculty in training courses on AI, conducted by Disease Investigation Section, Pune, Department of Animal Husbandry, Government of India, in the year 2006

Dr. AK Chakraberti, RO

Participated in Asia Pacific Conference on Medical Virology, New Delhi, Novermber 13-15, 2006.

Dr. (Mrs.) VA Potdar

Participated in Asia Pacific Conference on Medical Virology, New Delhi, Novermber 13-15, 2006.

Mr. PB Kulkarni

Participated in Asia Pacific Conference on Medical Virology, New Delhi, Novermber 13-15, 2006.

Ms. SR Waregaokar

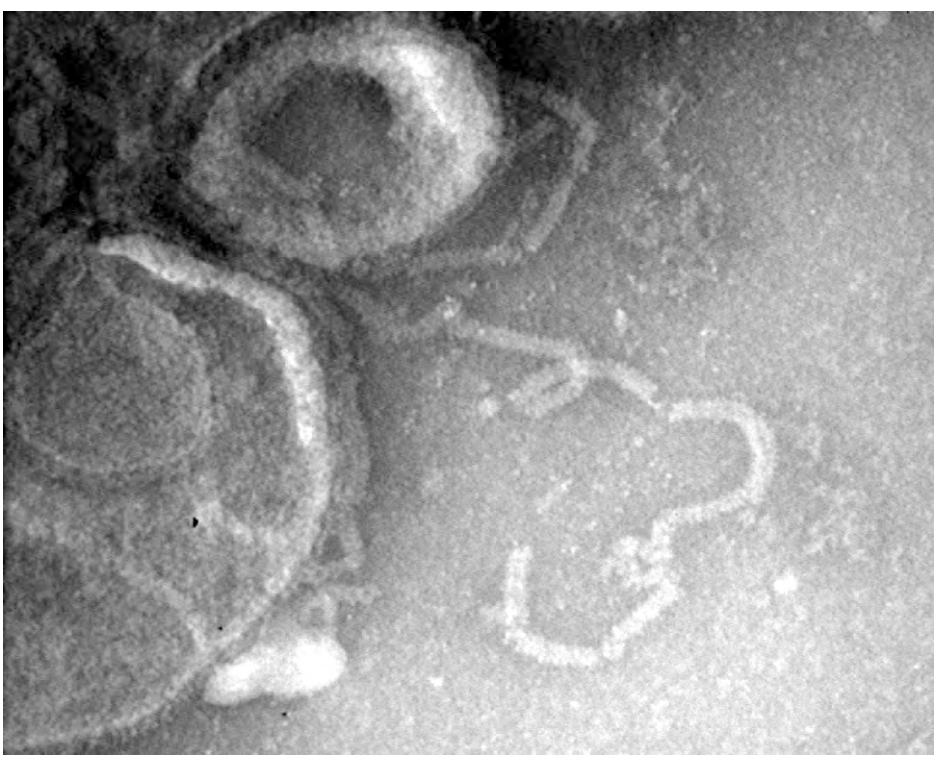
Participated in Asia Pacific Conference on Medical Virology, New Delhi, Novermber 13-15, 2006.

Ms. SS Naik

Participated in Asia Pacific Conference on Medical Virology, New Delhi, Novermber 13-15, 2006.


FIRE

Measles Virus



Measles Virus Group

Scientific staff

Dr. NS Wairagkar, MBBS,MD, Deputy Director, Group Leader
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Mr. LV Hungund, Technical Officer
Dr. (Mrs.) NJ Shaikh, Senior Technical Assistant
Mrs. VS Bhide, Senior Technical Assistant
Mr. RS Tomar, Technical Assistant
Mr. EG Bhargude, Technical Assistant
Mrs. CA D'Mello, Technical Assistant

Project : Measles Aerosol Vaccine

Project Staff

Dr. Deepak Karpe
Dr. Vani Kuppuswamy
Dr. Ashwini Kulkarni
Dr. Vaishali Ranpise

Project : Genotyping of Measles virus strains circulating in various parts of India.

Project Staff

Dr. Sarika Raibagkar
Ms. Deepika Khedekar

WHO-ICMR Measles Aerosol Vaccine Trial

Niteen Wairagkar, Naseem Shaikh, Neelakshi Kumbhar, Ashwini Kulkarni, V. Kuppuswami, S. Yadav, Laxman Hungund with Ashish Bavdekar, KEMH, Arun Risbud, NARI.

KEMH= KEM hospital

NARI= National Aids Research Institute

Introduction

Measles remain as a major global public health problem, specially in developing countries. The focus on measles as a major vaccine preventable disease has shifted the international health agenda in identifying and developing an integrated international initiative for global eradication of measles. This makes it imperative to test vaccine efficacy for measles using novel techniques to improve efficacy and provide larger community coverage. NIV has been identified as a major global partner to test the efficacy of measles vaccination through an aerosol route.

Summary of work done

This project with WHO-IVR is an International effort towards obtaining licensure for measles vaccine by aerosol route using three different aerosolizer devices. Extensive work has gone into preparation for this trial project, preparation of documents like, Measles Aerosol Vaccine Protocol, Investigator's Brochure, Informed consent forms, Patient Information Sheets, SOPs for various procedures etc. The Generic protocol converted into site-specific protocol, submitted to various committees like local ethics committees of three collaborators, DCGI, WHO ERC, ICMR, HMSC for approvals. All the regulatory approvals required for this trial were obtained. During April 2006, the study recruitment was started. Project staff was hired, GCP trained and screening for recruitment started in June 2006. The Good Clinical Practices (GCP) trainings and Ethics trainings were conducted. Pre-trial clinical monitor's visit and WHO evaluation of the site has been done. Actual recruitment of the subjects started in June 2006. 20 healthy, male from 18-35 years group has been screened and vaccinated with measles vaccine by aerosol route. We have completed 6 months of post-vaccination follow-up of adult subjects. Last visit follow-up for this group is scheduled in June 2007. The safety data was presented to PDG, WHO and DSMB. This group follow-up will be completed in June 2007. Preparation for next group (5-17 years) is going on. Lab evaluation by WHO- consultant expert Dr. David Brown, Director, HPA, UK was completed. International external audit of the lab and site by QMS Auditors was completed. David Featherstone, Global Measles Lab Coordinator visited the lab and appreciated the work. PRNT test

results were provided to all three sites in the project. Measles Group Infrastructure development plan is being prepared and implementation of GLP compliance is underway. This project is very labor-intensive and a publication may not emerge till the data is finally analyzed by all three groups and formally accepted by DCGI India and WHO-IVR, Geneva. The group is preparing documents for NABL accreditation.

WHO-NIBSC Collaborative study for Preparation of WHO III anti measles International Sera

Naseem Shaikh, Niteen Wairagkar

Summary of work done

WHO-NIBSC requested NIV Measles group to collaborate in this study in view of the expertise in PRNT for measles. This study was initiated to characterize the candidate sera pool for preparation and standardization as Measles International sera III. IS is usually used in PRNT to convert the titers in International Units and increase the comparability. 12 Global laboratories from 8 countries (4 National Control Labs, 4 Vaccine manufacturers, 4 Public health Labs), including NIV measles lab, were invited to carry out Measles assays on the blind coded sera panel supplied by NIBSC. The results from these 12 labs were analyzed to find out suitability of the Candidate pool as WHO III anti measles International serum. The study samples comprised the candidate (in coded duplicate) and three other lyophilized sera along with the current 2nd IS (66/202). Laboratories were invited to carry out assays by the methods that would normally be employed in that laboratory. Potencies for the candidate and other samples included in the study were calculated in IU's by reference to the existing 2nd IS. In the PRNT assays the potency for the candidate 3rd IS was found to be 2.87 IU/vial while for ELISA the mean potency was estimated at 5.37 IU/vial. NIV results were well received and were within the ranges as compared to other global labs. This is the major contribution towards International health.

WHO Measles Genotyping Project and MeaslesNetIndia

Niteen Wairagkar, Deepika Khedekar, Sarika Raibagkar, Laxman Hungund, R.S.Tomar, Naseem Shaikh with various MeaslesNetIndia collaborators

Summary of work done

Measles genotyping project ran very successfully this year with outbreak investigations of measles from various states. These outbreaks were investigated by MeaslesNetIndia - NIV initiated ICMR Measles Molecular surveillance network. MeaslesNetIndia was established last year in a startup workshop. Outbreak information is sought from various health agencies (Private & Government) &/ or from collaborators of MeaslesNetIndia. In the laboratory serology, Measles virus isolation using tissue culture methods and Sequencing N and H genes of different strains of measles was carried out. WHO-NIV Review workshop in December 2006 analyzed the progress made by the network.

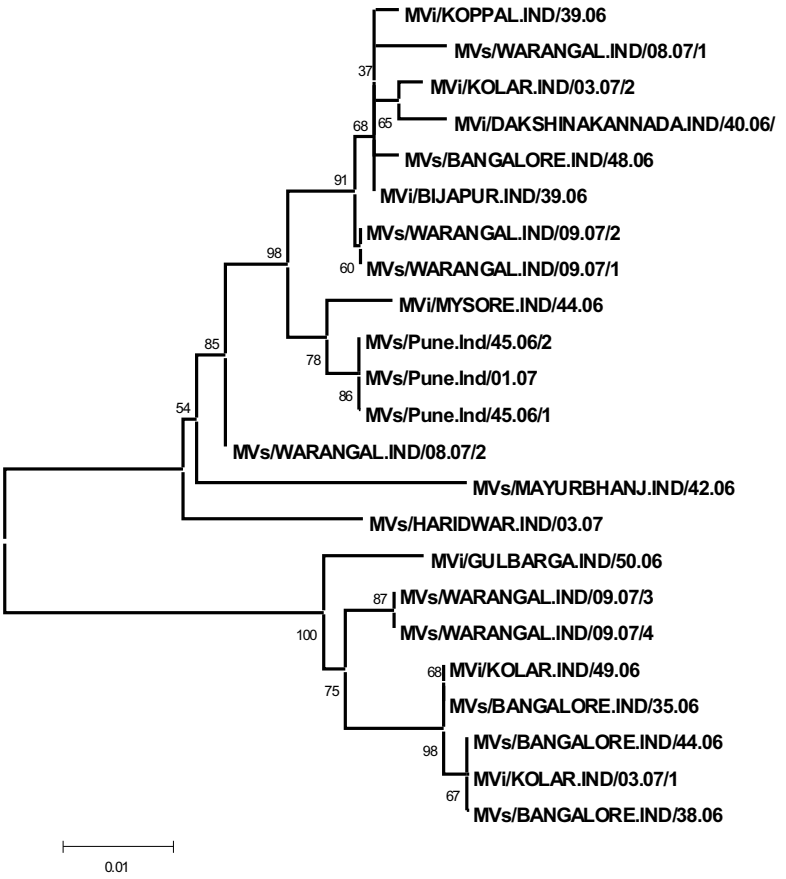
In the reporting year MeaslesNetIndia carried out measles outbreak investigations carried out in 11 states and genotyping studies from 8 states. All these outbreaks were serologically confirmed. Representative specimens were collected for serology and virus isolations. RT-PCR and sequencing studies were done to obtain N gene (456 bp) and full H gene (1854 bp) sequences. Measles genotypes D4 and D8 predominate majority of the states. D7 measles genotype was detected from three cities. Epidemiology data generated by the network indicated changing trends in Measles epidemiology, notably increase in measles cases in age above 5 years and measles cases in vaccinated children. Seasonality is also assessed and measles cases are occurring throughout the year in India though individual states have seasonality patterns. In conclusion, MeaslesNetIndia is a new model initiative by NIV involving various agencies for measles surveillance in absence of formal countrywide network.

Indian Measles Sequence database (IMSD) will be linked to WHO Global database and sequence data from all the geographic areas will be submitted to Global database. IMSD has now over 140 N gene sequences and 32 H gene sequences and numbers are increasing by each week.

Indian Measles Sequence database

Niteen S.Wairagkar, Deepika Khedekar

This database now has 140 N gene sequences and 32 full H gene sequences. Relevant epidemiological information is available and sequence cards are being prepared to make this database a searchable one.



Fig, 1:Measles N-gene Sequences from Eight states

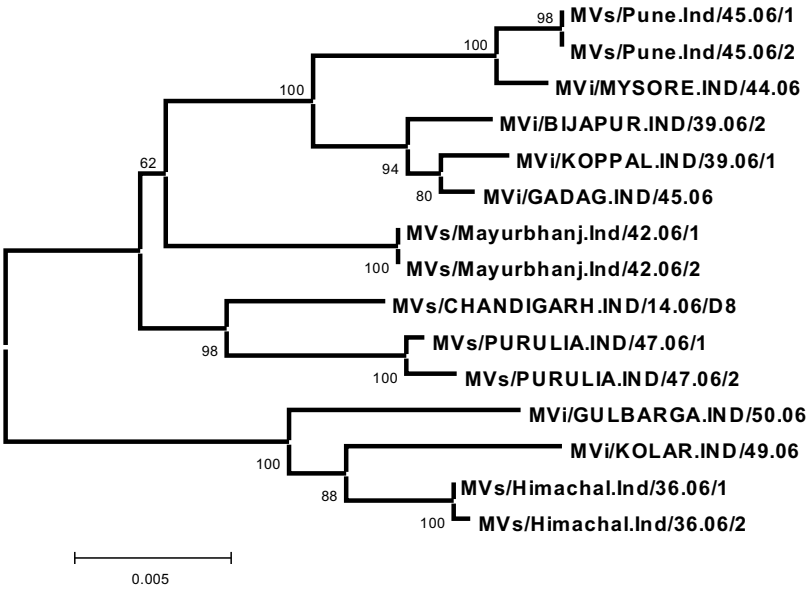


Fig. 2: Measles H-gene Sequences from Six states

Serological Studies on Measles Using Oral Fluid Specimens

Naseem Shaikh, Ayush Goyal, Arti Kinikar, Niteen Wairagkar
Collaboration with BJ Medical College, Pune

Serum is usually used in serosurveys for analyzing measles antibody prevalence for various uses. Oral fluid containing gingival secretions and salivary fluid is considered a suitable replacement in number of studies in west. In view of this we conducted a study with 100 pairs of serum and oral fluid specimens from a randomly selected group of children between 1-15 years. During sept-Nov, 2006, Oral fluid and serum samples were collected from 100 apparantly asymptomatic healthy children stratified into age groups of < one year, 1-5 years and > 5 years. IgG antibody prevalence was analysed using Commercial IgG capture ELISA (Microimmune) developed by Health Protection Agency, UK. The study showed concordence of 89% between serum and oral fluid IgG values and coefficient of correlation was 0.97 (Karl Pearson's). This study enphasized the importance of Oral fluid as replacement specimen for serum in large studies in India. The obvious advantage is the ease of collection of oral fluid even by non-technical staff.

Publications

Book Chapter :

Wairagkar Niteen. Acute Hemorrhagic Conjunctivitis. In: Current Ocular Therapy. Ed. Roy and Fraunfelder. ELSEVIER, UK. 2007.

Reports:

WHO/BS/06.2031/ 2006. Assisted in Preparation of report to WHO expert Committee on Biological Standardization. Report of collaborative study to assess the suitability of Replacement for second International Standard for anti-measles serum.

Papers:

01. Mohan A, Murhekar MV, Wairagkar NS, Hutin YJ, Gupte MD. Measles transmission following the tsunami in a population with a high one-dose vaccination coverage, Tamilnadu, India 2004-2005. BMC Infect Dis 2006, Sept 19; 6(1): 143.

Proceedings Publications

1. Wairagkar NS et al. MeaslesNetIndia- New model for measles surveillance in India. Proceedings of IMED (International meeting on Emerging Diseases and Surveillance), Vienna, Feb 2007.
2. Wairagkar NS, Raibagkar SD, Vashishtha VM, Khedekar DD, Shaikh NJ. Genotyping of measles strains circulating in Uttar Pradesh, India, VED 2, Proceedings for Asia Pacific Congress of Medical Virology, New Delhi, Nov, 2006; pp 110.
3. Wairagkar NS, Sugunan AP, Khedekar DD, Raibagkar SD. VED 1, Proceedings for Asia Pacific Congress of Medical Virology, New Delhi, Nov, 2006; pp 110.
4. Vaidya SR, Raja D, Mahadevan A, Wairagkar NS, Ramamurthy N, Shankar S. Measles genotype D7 is indigenous in India. VED 3, Proceedings for Asia Pacific Congress of Medical Virology, New Delhi, Nov 2006; pp 111.
5. Jagtap M, Wairagkar NS, Sathe PS, Thakare JP. Identification of Dengue virus serotypes by IgM Capture ELISA. D 22, Proceedings for Asia Pacific Congress of Medical Virology, New Delhi, Nov 2006; pp 90.
6. Hundekar S, Wairagkar NS, Singh a, Sathe PS Investigation of an outbreak of acute encephalitis in Rae Bareilly. Arbo 16, Proceedings for Asia Pacific Congress of Medical Virology, New Delhi, Nov, 2006; pp 105.

Participation in meetings, workshops

Dr. Niteen Wairagkar

PDVI field consortium meeting at Bangkok, March 2007.

International Meeting on Emerging Diseases and Surveillance (IMED) at Vienna, Austria, February 2007.

IVI International Seminar on "New Vaccines for Improving Health and Economic Development in Developing Countries", Kolkata, February 2007.

WHO-IVR Product Development Group meeting on Aerosol vaccine, Kolkata, January 2007.

WHO-NIV workshop on Review of Molecular surveillance network for measles in India, December, 2006

WHO-IVR "Onsite GCP and Device Training for Clinical Investigators at NIV, Pune for Measles Aerosol Vaccine Project" at NIV, Pune, 3-5 April 2006.

WHO-IVR "Onsite GCP and Device Training for Clinical Investigators at NICED, Kolkata, for Measles Aerosol Vaccine Project" at Kolkata, 5-7 April 2006.

Joint State Conference of IAPSM & IPHA, Savangi-Meghe, 29 -30th Dec 2006 and delivered MC Pathak Oration.

National Disaster Management Authority (NDMA) Conference on "Mass casualties and Medical Preparedness", New Delhi, 22-23rd August 2006.

Indian Academy of Pediatrics conference (PEDICON) in January 2007.

Mr. Sunil Vaidya

WHO-IVR "Onsite GCP and Device Training for Clinical Investigators at NIV, Pune for Measles Aerosol Vaccine Project" at NIV, Pune, 3-5 April, 2006.

WHO-NIV workshop on Review of Molecular surveillance network for measles in India, December, 2006.

Asia Pacific Conference on Medical Virology, New Delhi, December, 2006

Dr. Naseem Shaikh

WHO-IVR "Onsite GCP and Device Training for Clinical Investigators at NIV, Pune for Measles Aerosol Vaccine Project" at NIV, Pune, 3-5 April, 2006.

WHO-IVR "Onsite GCP and Device Training for Clinical Investigators at NICED, Chennai, for Measles Aerosol Vaccine Project" at Kolkata, 5-7 April, 2006.

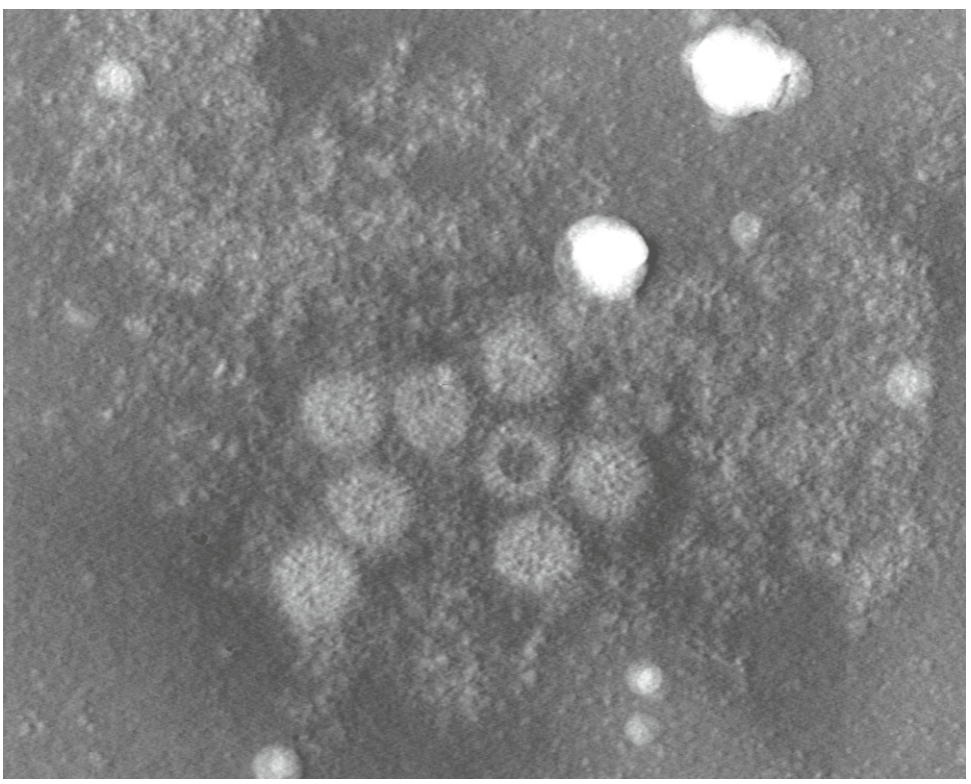
Global Vaccine Research Forum meeting in Bangkok, 2006

Measles Group participated in

WHO-IVR "Onsite GCP and Device Training for Clinical Investigators at NIV, Pune for Measles Aerosol Vaccine Project" at NIV, Pune, 3-5 April, 2006.

WHO-NIV workshop on Review of Molecular surveillance network for measles in India, December, 2006.

Rota Virus



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Epidemiological Studies on Rotaviruses

Shobha Chitambar

Hospital based surveillance of rotavirus disease and strains among children

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 non-typeable 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 290 children <5 yrs of age, hospitalized for diarrhea. Nearly 39.6% specimens were detected positive for rotavirus by ELISA. Among these specimens, 87.8% were typed for VP7 (G) (Fig. 1A) and 93% were typed for VP4 (P) (Fig. 1B) genes. Both G and P types were established in 82.6% of the specimens by multiplex PCR. Each of the two common rotavirus types G1P[8] and G2P[4] represented 31.5% and 34.7% of the strains respectively while none of the specimens showed presence of other two common types-G3 P[8] and G4P[8]. G9P[8] and G12P[8] were detected at a frequency of 9.9% and 1.9% respectively. G and P types in uncommon combinations and in mixed infections represented 12.6% and 8.4% of the strains respectively. Nearly 0.8% of the strains remained non-typeable.

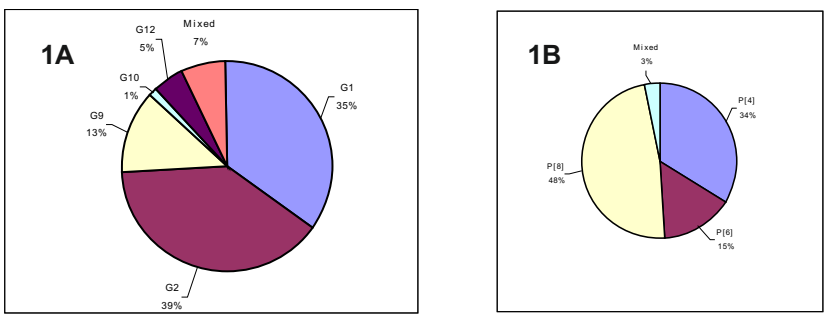


Fig. 1A & B. Distribution of rotavirus strains among typeable specimens

Hospital based surveillance of rotaviruses was also carried out in acute diarrhea patients from Aurangabad (n=122) and Nagpur (n=94) cities of northern Maharashtra. Rotavirus positivity was detected in 11.5% and 25.5% patients respectively. G12 strains of rotavirus were detected in 50% of the cases from Aurangabad.

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

VS Tatte, SD Chitambar

Although Rotavirus infections in adults are milder than those in children, deaths due to rotavirus have been reported. In India, limited studies conducted in adults indicate 5-7% prevalence of rotavirus diarrhea. However, role of rotaviruses as a pathogen in adults has long been under appreciated.

Objective

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

Work done

Fecal specimens collected during 1993-96 and 2004-06 respectively from hospitalized (n=1313) and OPD (n=183) cases of acute gastroenteritis that occurred in adolescents and adults were examined for rotaviruses. The two sets showed presence of group A rotavirus antigen in 5.7% and 4.9% specimens respectively in antigen capture ELISA. Among ELISA positive specimens 78.6% and 85.7% were G and P typed respectively by multiplex PCR (Table). Both G-P types were determined in 74.7% and 66.7% specimens at two time periods respectively. In early 1990s, G2P[4] was predominant (30.4%) followed by G1P[8] (17.9%), G3P[8] (5.4%) and G4P[8] (3.6%), while in 2004-06, circulation of only G2P[4] (33.3%)was detected (Table). G9 serotype detected in both the time periods prevailed in unusual combinations with P types-P[6], P[4] P[6] and P[4] P[8]. Thirty percent specimens showed dual / triple infections of G and P types during the period of study. Infections with uncommon G-P type combinations were 5-16% (Table 1). Non-typeable G and P types appeared to be on the rise.

Table 1. Year wise distribution of rotavirus strains among adolescent and adult cases of Gastroenteritis

Types	Year (1993-96) n=56 (%)	Year 2004-06 n=6 (%)
Common:		
G1P[8]	10/56 (17.85)	0/6 (0)
G2P[4]	17/56 (30.35)	2/6 (33.33)
G3P[8]	3/56 (5.35)	0/6(0)
G4P[8]	2/56 (3.57)	0/6(0)
G9P[6]	4/56 (7.14)	0/6(0)
Uncommon	3/56(5.35)	1/6(16.66)
Mixed G-P types	17/56 (30.35)	3/6 (50)

Full length genome sequencing and characterization of rotavirus strains

SD Chitambar, PR Fadnis

Rotavirus is an important agent causing diarrhea in children as well as in adults. The virus genome consists of 11 segments of double stranded RNA which code for six structural and five non-structural proteins. Currently, complete genome sequence data is available for few rotavirus strains originated from human, monkey, cattle, rabbit and bird. Full genome sequencing of human rotavirus strains would be useful to characterize unusual strains detected in human infections and to analyze the role of each gene segment in causing restriction and virulence in different hosts.

Objective

To characterize entire genome of rotavirus strains.

Work done

The rotavirus strain indicating unusual combination of VP7 and VP4 genes i.e. G12 and P[6] was undertaken for full length genome sequencing. The sequences obtained for VP7 and VP4 genes showed respectively 97.60% - 97.90% and 95.70% - 99.0% nucleotide identities with strains from Belgium, Philippines, Bangladesh and UK reported very recently.

Molecular characterization of rotavirus strains adapted to cell culture and effect of rotavirus infection on cytoskeleton

Ritu Arora, SD Chitambar

Rotaviruses, members of the family Reoviridae, are the most common cause of severe diarrhea among children. Characterization of rotavirus genes especially of VP 7 and VP-4 directly 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 strain from acute diarrhea patient.
To study the genomic changes in rotavirus during adaptation to tissue culture.

Work done

The project was initiated by inoculating commonly circulating rotavirus strains, G1P[8] in MA104 cell line. The infected cells were observed microscopically to monitor cytopathic effect (CPE). The virus was detected at each passage level in antigen capture ELISA. However, clear CPE was observed on 5th day post infection at passage level 5 (Figure 2 - A and B).

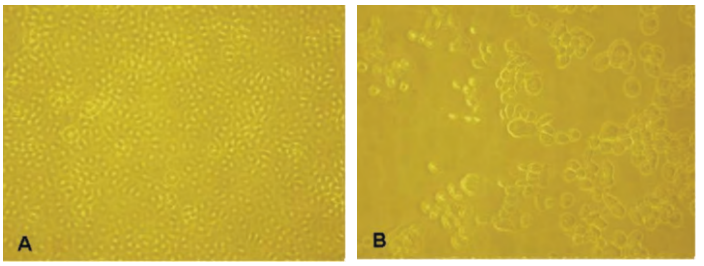


Figure 2 : Phase-contrast micrographs of MA-104 cells: (A) Normal cells and (B) Cells infected with rotavirus strain at 6th passage (Magnification x 400)

Genomic Characterization of rotavirus strain from fecal specimen undertaken fro isolation and adaptation in cell culture is being carried out.

Detection of rotavirus serotype specific antibodies in human sera

PG Ray, Shilpa Bhalla, SD Chitambar

Rotaviruses are recognized as the most important etiologic agents of childhood diarrhea worldwide. Widespread distribution of rotavirus is indicated by universal acquisition of serum antibodies to rotaviruses at an early age. High prevalence of rotavirus antibodies in the adult life suggest that repeated infections occur. Rotavirus infection in adults is relatively mild and presence of serum antibody may be indicative of past infection with rotavirus.

Objectives

To detect rotavirus serotype specific antibodies in serum samples of healthy individuals

Work done

Serum samples collected from 33 healthy individuals without any topical history of diarrhea and stored at -20ºC were tested for human G1-G4, G9 and animal G6 (bovine) and G3 (simian) rotavirus serotype specific antibodies by using blocking ELISA. Specific antibodies to human rotavirus serotypes G1-G4 and G9 were detected in 54.5%, 60.6%, 93.9%, 100% and 3.03% respectively, whereas presence of antibodies to animal serotypes G6-bovine and G3-simian was noted in 78.8% and 93.9% individuals respectively. The results of 15 sera were compared with that of the conventional tissue culture and ELISA based neutralization assay (Fig. 3). Proportion of positivity in both the assays was detected to be similar. The range of titres was 1:50-1:100 for G1 and G2; 1:50-1:400 for G3, G4 and simian G3, 1:50-1:200 for G6 bovine and 1:50 for G9.

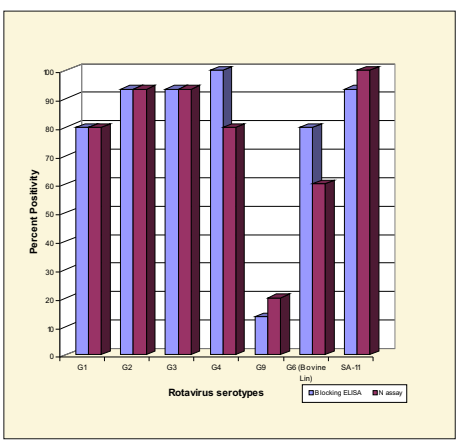


Fig. 3 Comparison of anti-rota antibody positivity in Blocking ELISA and Neutralization (N) assay

Preparation of egg yolk antibodies against human rotaviruses

Manika Burgohain, GS Dhale, SD Chitambar

Eggs are a complete diet for the developing embryo and a supplement for the first few days of life of 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

Work done

Three groups of SPF hens, each consisting of 7 birds were immunized against human rotaviruses - HRV1, HRV2 and HRV9 at 100 µg/ dose using Freund's complete or incomplete adjuvant at biweekly interval. Birds from all 3 groups generated anti-rota antibodies which showed titres in the range of 1:50,000-1:1,00,000 (Fig. 4). Eggs were collected from the birds and processed for purification of IgY, protein estimation and ELISA titration. The protein content of the purified IgY was in the range of 2.4-3.5 mg/ml and antibody titres varied from 1:64,000 to 1:256,000 in various lots (Fig. 4)

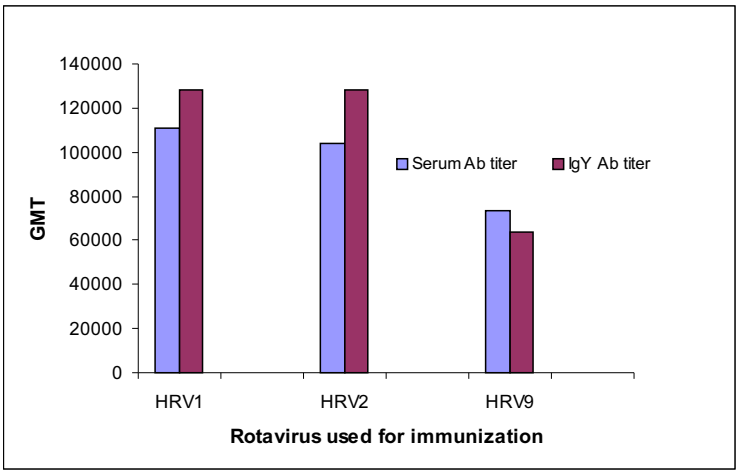


Fig. 4. Serum and Egg yolk antibody (IgY) titers in birds immunized against rotaviruses

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

SD Chitambar, V Goplakrishna

Acute gastroenteritis is one of the most common diseases in humans and continues to be a significant cause of morbidity and mortality worldwide. Viral gastroenteritis is a common disease. 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, 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, Entero, Picobirna and Toro viruses in sporadic infections and outbreaks of gastroenteritis in India.

To determine the age stratified sero prevalence of neutralizing antibodies to the non-rota enteric viruses in gastroenteritis patients and healthy individuals.

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

Preeti Chhabra, SD Chitambar

One hundred and ninety two fecal samples collected from children suffering from acute diarrhea were screened by RT-PCR using RNA polymerase region primers specific for genogroups (G) I and II of noroviruses. Twenty four (12.5%) samples showed positivity to GII noroviruses by PCR and sequencing while none was positive for G I. Phylogenetic analysis placed 15 strains in GII- subgroup (SG) 4 cluster, 4 strains in GII-SG2 cluster, 1 strain in GII-SG3 cluster, 1 strain in GII-SG1 cluster and 3 strains closer to newly identified rotavirus genetic cluster GIIb (Fig. 5).

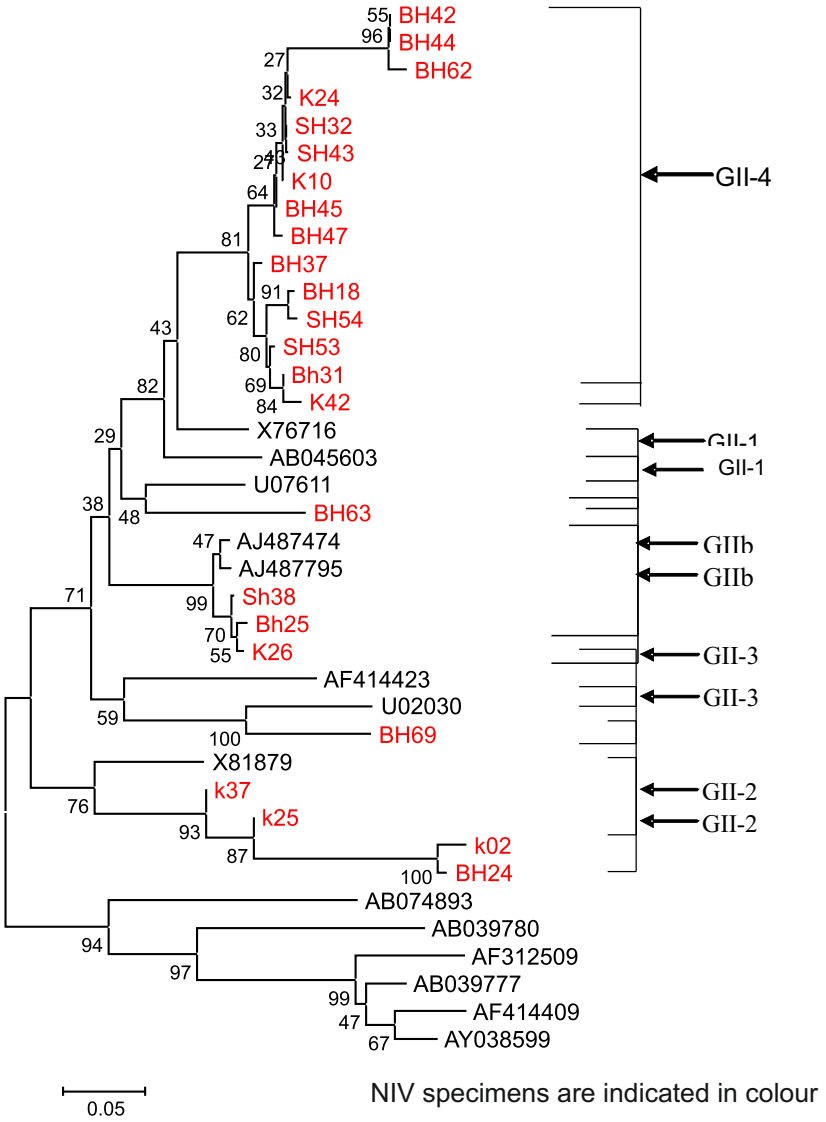


Fig. 5. Phylogenetic tree indicating relationship between Pune strains and reference strains of GenoGroup I & II. NIV specimens are indicated in colour.

Hospital based detection and characterization of enterovirus strains in acute gastroenteritis

Pooja Patil, SD Chitambar, V Gopalkrishna

A total of 95 fecal specimens from infants and children with acute diarrhea were collected from Aurangabad, northern Maharashtra and subjected to RT-PCR for enterovirus detection using primers from 5'NCR. Twenty one samples (22.1%) were tested positive for enterovirus genome. Sequence analysis indicated presence of Cox-A in 12 (56%), Cox-B in 2 (10%), HEV-A in 3 (14%), HEV-D in 1 (5%) and Echo-13 in 1 (5%).

Hospital based detection and characterization of enteric-adenovirus strains in acute gastroenteritis

Harsha Verma, SD Chitambar, V Gopalkrishna

One hundred fecal specimens from infants and children suffering from acute diarrhea were collected from Aurangabad, northern Maharashtra and tested for adenovirus by PCR using Hexon region primers. Adenovirus DNA was detected in 7% of specimens. Sequencing of PCR products revealed adenovirus 40/41 (6), AD-31 (1) strains in gastroenteritis patients.

Additional Studies

Investigation of an outbreak of acute gastroenteritis

SD Chitambar, V Gopalkrishna

An outbreak of gastroenteritis occurred in March, 2006 in Southern Mumbai. Seventy one fecal specimens were collected from hospitalized cases of diarrhea during an outbreak. Thirty fecal specimens were also obtained from asymptomatic individuals residing in the gastroenteritis affected areas 2 months after the outbreak. All specimens were subjected to PCR using primers specific to Group A Rotavirus (VP6 gene), Group B Rotavirus (gene5), Norovirus (genogroup I and II), Enterovirus (5' NCR), Adenovirus (Hexon Region) and Astrovirus (ORF-1a). Among outbreak cases Group A rotaviral RNA was detected in 32.85% of fecal specimens and none of the samples contained group B rotavirus RNA (Fig. 6). Norovirus RNA specific to genogroup II was detected in 12.6% specimens while presence of enterovirus RNA was noted in 31.4% samples. Adenovirus DNA was detected in 14.3% of the specimens (Fig. 6). Co-infections of rota- and noro-,rota- and entero and rota and adeno-viruses were detected at variable levels (3.9%-7.8%). None of the samples was found positive

for astrovirus. Rota-, Adeno and entero- virus infections in children <10 yrs were higher as compared to that of adults while norovirus infection was higher in adults.

Rota viral RNA was detected in 26.6% fecal specimens of asymptomatic population. None of the fecal samples contained Noro, Adeno and Astro viral RNA and Adeno viral DNA. Enteroviral RNA could be detected in 6.6% of the samples. PCR products derived from representative specimens were sequenced and identity of the respective viruses was confirmed. Rotavirus VP6 gene sequence identity was >90% with simian (n=4), bovine (n=3) and human (n=11) strains. The Norovirus strains (n=9) showed 93% nucleotide identity with that of Denmark strain. Among enterovirus positive specimens, EV-89/76(n=5), CA(n=9), EV90(n=2), Human entero mussles (n=3) and Poliovirus (n=3) were detected while the specimens positive for adenovirus DNA showed nucleotide sequences specific for type F in 7, type A in 2 and type D in one.

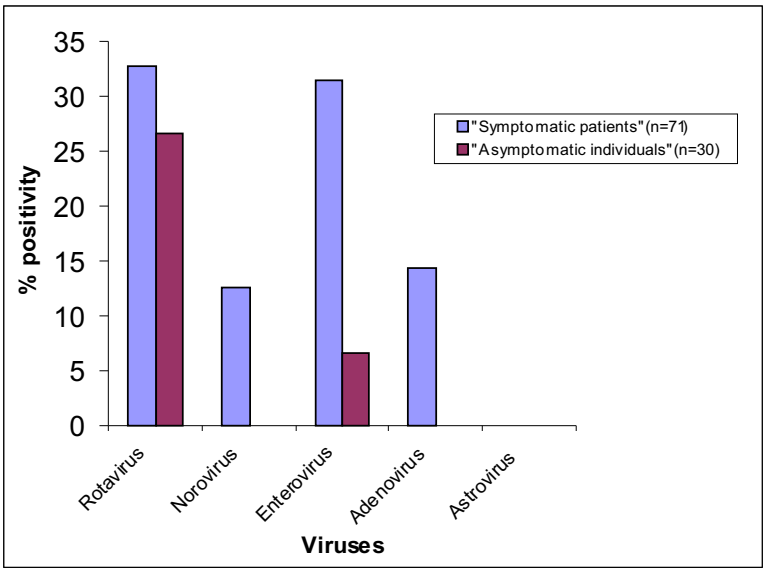


Fig. 6. Distribution of enteric viral pathogens in acute gastroenteritis patients and asymptomatic individuals

Rapid detection of rotavirus in acute diarrheal patients

SD Chitambar, Prachi Fadnis

Agarose gel electrophoresis was standardized for detection of rotavirus RNA migration pattern by optimizing concentration of agarose in gel, voltage, time for running the RNA on gel and quantity of RNA. Agarose gel at 1.2% and electrophoretic run at 70 volts and for 120 mins were found to be suitable. The method is useful for screening of ELISA positive fecal specimens for detection and analysis of RNA migration pattern for group A rotaviruses and also for ELISA negative specimens for detection of non-group A rotaviruses.

Sequence and structural analyses of inner capsid protein (VP6) of chicken rotavirus strain CH2

Dr. S.D. Chitambar, Manika B

Bioinformatic Group (Collaborator)

The inner capsid protein VP6 of group A rotavirus is known to possess group and subgroup (SG) epitope specificities. Based on immunological characteristics of VP6 rotaviruses are categorized into seven groups (A-G) and further classified as SGI, SGII, SGI/II and non SGI/II. Group A avian rotaviruses have a unique VP6 that is antigenically different from its mammalian counterpart. The lack of information on the VP6 gene of group A chicken rotavirus strain, CH2, at the sequence and structure level was a major motivation for this work. Sequencing of the complete cDNA of the VP6 gene of CH2, revealed a nucleotide (amino acid) identity that varied from 78.32-98.58% (86.40-98.24%) when compared with other avian rotaviruses. Regardless of its host origin similarity, CH2 VP6 showed a close sequence homology (1.763.02% amino acid divergence) with avian rotaviruses other than that of chicken origin viz. turkey and pigeon. Based on the knowledge that the subgroup specific epitopes are conformational and present only in the trimeric form of VP6 (Lopez et al., 1994), a three dimensional structure of CH2 VP6 protein was modeled from crystallographic coordinates of VP6 protein of bovine rotavirus RF and the conformational epitopes, that might be critical for antibody binding were predicted. Detailed description of the modeling and conformational epitope mapping of CH2VP6 is narrated in Bioinformatic section.

Publications

1 Awachat PS, Kelkar SD. Dual infection due to Simian G3 human reassortment and human G9 strain of rotavirus in a child and subsequent spread of serotype G9 leading to diarrhea among grand parents. *J Med Virol* 2006; 78:134-38.

2 Chitambar SD, Fadnis RS, Joshi MS et al. Case report: Hepatitis A preceeding Guilliane Barre syndrome. *J Med Virol* 2006; 78:1011-14.

3 Chitambar S, Joshi M, Lole K, Walimbe AM, Vaidya S. Cocirculation of and coinfections with hepatitis A virus subgenotypes IIIA and B in patients from pune, Western India. *Hepatol Res* 2007, 37 : 85-93.

4 Kelkar SD,Zade JK,Dindokar AR, Dhale GS,Vaishya SS. Outbreak of diarrhoea in Daman and detection of group B rotavirus from three adult cases (Correspondence). *National Medical Journal of India* 2007, 132 (1) 337-341

5. Ray PG, Kelkar SD, Walimbe AM, Biniwale Vaishali, Mehendale Savita. Rotavirus immunoglobulin levels among Indian mothers of two socio-economic groups and occurrence of rotavirus infections among their infants up to six months. J Med Virol 2007 (March), 79(3): 341-349.

Participation in meetings, workshops

Dr. SD Chitambar

Meeting of the Indian Rotavirus Working Group held on 25th June, 2006 at Kolkata, West Bengal.

. Task Force Meeting on Hospital Based Surveillance Network for Rotavirus Disease and Strains, organized by ICMR at Mahabalipuram, Chennai on 12th Sept. 2006.

Seventh Asia Pacific Congress on Medical Virology (APCMV) at India International Centre, New Delhi, Nov 13-14, 2006.

Meeting of the Indian Rotavirus Working Group held on 13th January, 2007 at Powai, Mumbai.

Annual meeting held at ICMR HQS on 19th Feb., 2007 to present the progress report of the project under Genomics and Medicine.

17th APASL Conference 2007 'Viral Hepatitis and Hepatocellular Carcinoma in Asia' at Kyoto, Japan, 27th-30th March 2007.

Dr. Gopalkrishna V

Seventh Asia Pacific Congress on Medical Virology (APCMV) at India International Centre, New Delhi, Nov 13-14, 2006.

Dr. PG Ray

. Sixth Annual Meeting of the Federation of Clinical Immunology Societies (FOCIS 2006), June 1-5, 2006 in San Francisco, California.

WHO Advanced Course on Immunology Vaccinology and Biotechnology in Lausanne, Switzerland, from 5th Sept. 2006 to 20th Oct. 2006 (Dr. PG Ray).

Seventh Asia Pacific Congress on Medical Virology (APCMV) at India International Centre, New Delhi, Nov 13-14, 2006.

Mrs. MS Joshi

VIIth 'Asia Pacific Congress on Medical Virology'(APCMV) at India International Centre New Delhi, Nov 13-14, 2006\

17th APASL Conference 2007 'Viral Hepatitis and Hepatocellular Carcinoma in Asia' at Kyoto, Japan, 27th-30th March 2007.

Mrs VS Tatte

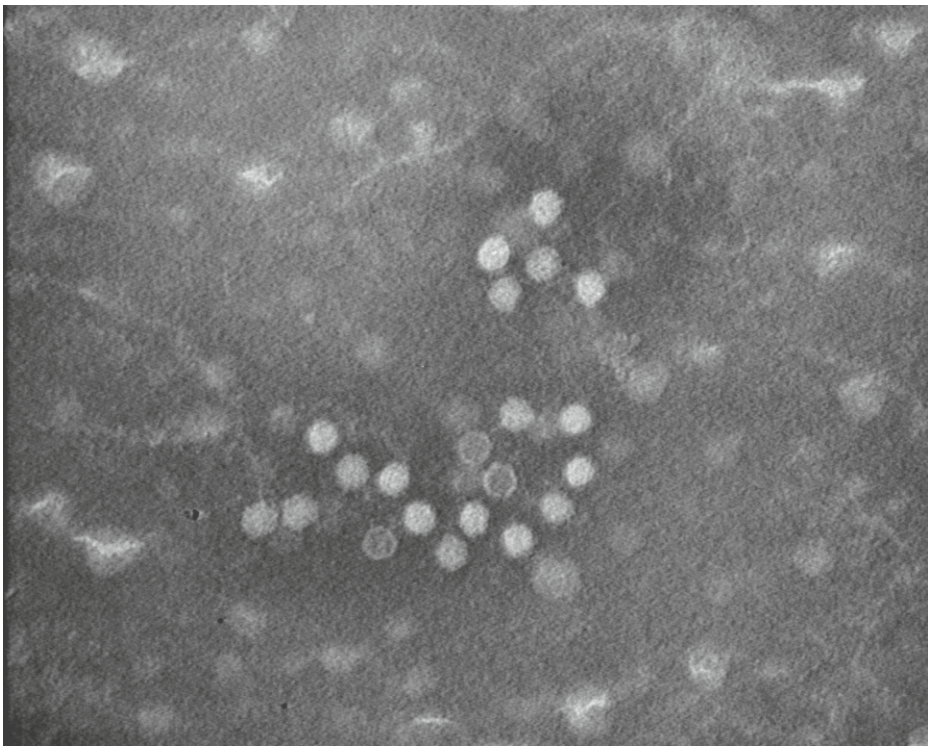
. Seventh Asia Pacific Congress on Medical Virology (APCMV) at India International Centre, New Delhi, Nov 13-14, 2006.

Ms Pooja R. Patil

. Seventh Asia Pacific Congress on Medical Virology (APCMV) at India International Centre, New Delhi, Nov 13-14, 2006.

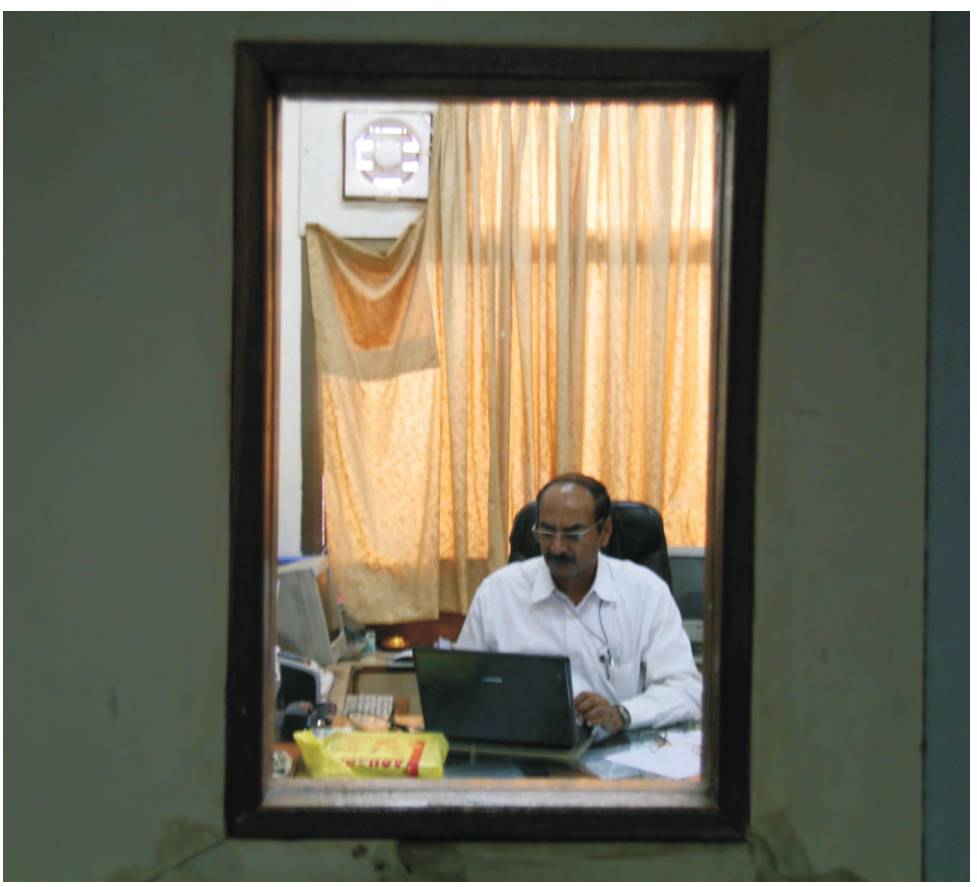
Mr. Fadins R

Seventh 'Asia Pacific Congress on Medical Virology'(APCMV) at India International Centre New Delhi, Nov 13-14, 2006



Negative stained transmission electronmicrograph of Norwalk virus

HCL & VRG



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Studies on genotyping of Kyasanur forest disease virus

DT Mourya, R Mehla

Introduction

Kyasanur Forest Disease virus (KFD) is a human hemorrhagic flavivirus unique to the Shimoga district of India. We continue to report sequence analysis studies to examine evolutionary trends and phyolgeny of this virus.

Objectives

Genotyping of KFD isolates (using NS5 and E gene) to understand variation in the strains isolated during last 3 decades.

Work done

Phylogenetic analysis of KFD virus

Fifty strains of KFD virus were chosen for the phylogenetic study from ticks, monkey and human . Using different RT-PCR conditions and primers all three genes of this virus amplified and sequenced. The sequences were assembled using Kodon software version 2.1 and curated manually. Multiple sequence alignment was done using ClustalW and MEGA software. Colinearized tree was constructed using in frame structural and NS5 gene of 50 isolates. The tree construction was done by MEGA software using maximum parsimony method with 1000 bootstraps pseudoreplicates. Kimura 2-parameter was used as substitution model wherein substitutions included both Transitions and transversions and the pattern among lineages were homogenous with uniform sites. There was 98.8 to 100% nucleotide and 99.5 to 100% amino acid similarity among KFDV isolates. A recent virus isolate, A106 showed 98.8% nucleotide and 99.5% amino acid identity with earlier isolates. Comparison of average nucleotide p distance of various isolates with respect to time reveals that the nucleotide distance is constantly increasing and probably the mutations are gradually getting accumulated over 50 years. Isolates from monkeys, man and ticks are in the same clade showing the conservation of degree of variation in various hosts (Fig. 1).

Isolation of KFDV from the patient's serum sample

Serum sample of the patient's (n=24) were obtained from Karnataka state laboratory, Bangalore. These were considered positive on the basis of the clinical features of the patients and appearance of sickness in infant Swiss albino mice after ic inoculation of serum. Attempts were made to isolate virus from these samples using *Vero E6* cell line so that the sequencing of the isolates done to see genetic changes in recent circulating virus in India. One of the isolate (A106)

showed CPE at the 3rd passage level and also found positive by RT-PCR was passaged into mice by ic route. Mice showed sickness on 3rd PID (Table-1).

Table 1: CPE in the Vero-E6 cell line with different clinical samples

#	Serum Sample	Year	Passage levels	Status/ comment
1	A1063	2005	1st	No CPE
2	A834	-Do-	1st	-Do-
3	A1137	-Do-	2nd	3rd (under progress)
4	A732	-Do-	2nd	3rd (under progress)
5	A106	2006	3rd	Showed CPE and at 3rd passage it was RT PCR positive. Sequencing work undertaken (under progress)
6	A131	-Do-	4th (under progress)	Showed CPE but was negative by RT PCR at 3rd passage so taken for 4th passage.
7	A122	-Do-	1st	No CPE
8	A179	-Do-	1st	-Do-

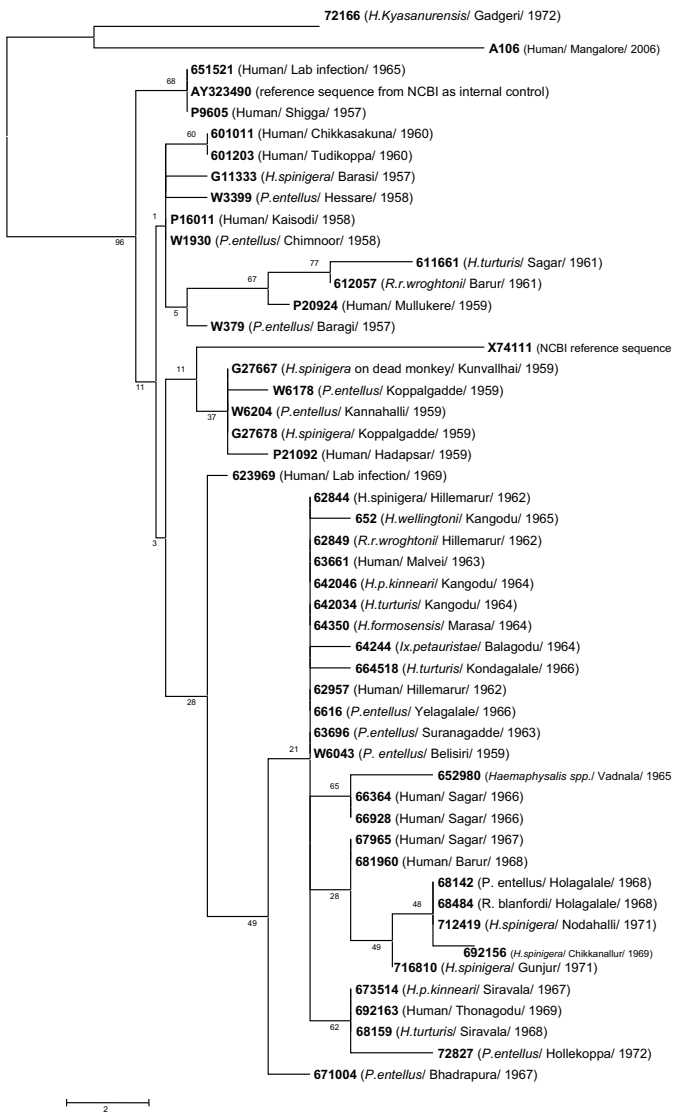


Fig. 1: Phylogenetic tree generated from colinearized data of both partial structural and nonstructural genes of 1281 bp.

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

DT Mourya, PD Yadav, PV Barde , RS Jadi, R Mehla.

Objective

In continuation with efforts of the previous year, further optimization for developing a diagnostic method for laboratory confirmation of KFD infections was carried out.

Work done

Preparation of KFD antigen for diagnostic tests

Twenty-five Swiss albino infant mice groups were inoculated with P9605 strain, brains were harvested from sick mice and 20% stock was prepared in BAPS, inactivation was done using 1:1000 formalin, at 4o C for 5 days. Antigen inactivation was checked by inoculation into infant Swiss albino mice brain (1 group), which was observed for sickness till 21 days. No sickness and mortality was observed.

Development of MAC ELISA

Immunization was carried out in adult guinea pig and Swiss albino mice against KFDV W1930 strain. The inactivated virus was used for first dose followed by 4 doses of live virus W1930. The immune serum obtained from mice was used to test by ELISA for its activity to pickup KFD antigen. Titration of immune serum was done by ELISA. The KFDV antigen and immune serum was diluted from 1:10 to 1:400 with appropriate negative controls (normal mice brain suspension and normal mouse serum). Goat anti mouse HRP (Sigma) was used as a probe in dilution of 1:10000. The 1:100 dilution of Antigen and 1: 200 dilution of immune serum was giving P/N ratios above 3 (results mentioned below in graphical form Fig. 2), hence, these dilutions were further tested. A preliminary trial for MAC ELISA was done to detect the IgM antibody from clinical samples. Results showed P/N ration between 2 and 3 further work to standardize the test for clinical samples is being done.

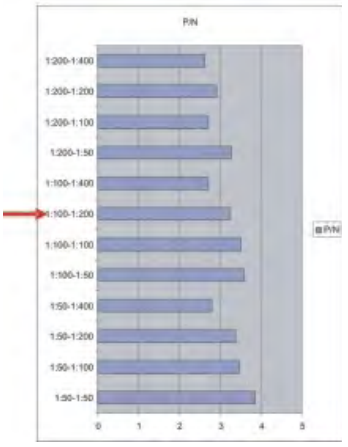


Figure 2: Graph showing different P/N ratios when different dilutions were used for ELISA the arrow indicates the dilution we will be using in further development

Development of Taq Man reverse transcriptase (RT-PCR) assay for rapid diagnosis

TaqMan assay for specific and rapid diagnosis of KFD virus is developed in collaboration with the Centers for Disease Control and Prevention, Special Pathogen Branch, Atlanta, USA. Three sets of primers and probes were designed for KFD virus using data bank sequence from NS-5 gene. Probes were labeled with FAM at 5' ends with BHQ (black hole quencher) 3' ends. The Ct values obtained from the TaqMan assay using serially diluted RNA showed the successful application of this assay for the detection of KFD virus. This assay could detect minimum 38 RNAs copy. Satisfactory Ct value was obtained for W-1930 strain. Viral RNA dilution till 10-8 dilutions could be detected.

Molecular markers for identification of some important mosquito vectors in India and their genetic variabilities in different geographical populations

DT Mourya , Y Shouche*, Lt Col Raj Kumar
* National Center for Cell Sciences Pune

Objectives

The main objective was to develop base line information for molecular and biochemical markers for the mosquito vectors *Aedes aegypti*, from various geographical areas in the country.

Work done

A total of 43 samples have been analyzed till date using RAPD-PCR and mitochondrial cytochrome b gene respectively. Results suggest that if these two techniques used in combination work better for understanding relatedness of various geographical populations of the vector species (Fig3& Fig-4).

Fig. 3: Phylogenetic analysis of the partial cytochrome-b gene sequences obtained from various geographical populations of Aedes aegypti from India.

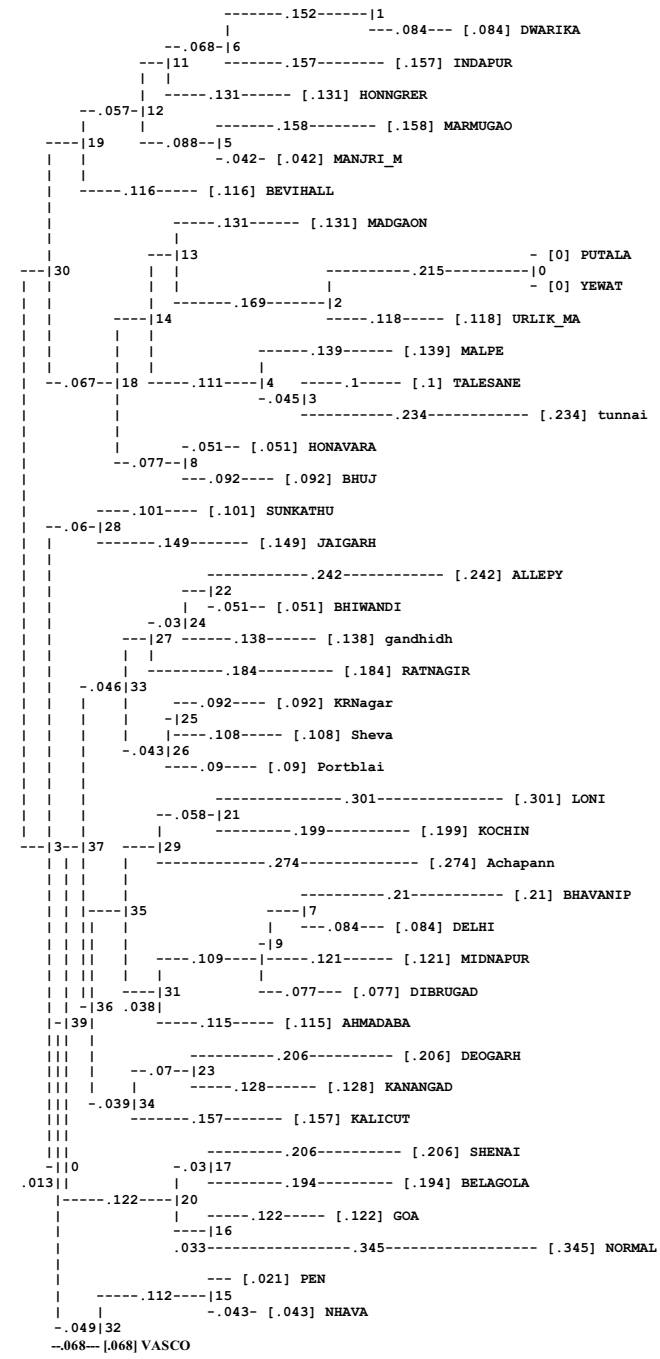


Fig. 4: Dendogram showing populations of *Aedes aegypti* from different geographical regions using random amplification of polymorphic DNA (RAPD) polymerase chain reaction (PCR)

Development of serological and molecular diagnostic facility for Hantaviruses

P.D Yadav, D.R Patil, DT Mourya

Hantaviruses are of major global public health importance due to their high morbidity and mortality in humans. Timely, rapid and accurate laboratory tests are crucial for confirmation of suspected hantavirus infection.

Objectives

To develop laboratory diagnosis for pathogenic hantaviruses

Work done

Nested RT-PCR

Nested RT-PCR standardization was done to detect a small fragment of Nucleocapsid gene and glycoprotein gene of Murinae group of Hantavirus by nested RT-PCR (Fig-5 and 6). To standardize the assay as a positive control, gamma-radiated Seoul virus used, obtained from CDC, Atlanta. Further one more conserved set of primer used to standardize nested RT-PCR based on the RNA polymerase gene, which can detect all three groups of Hantaviruses (Murinae, Arvicolinae and Sigmodontinae).

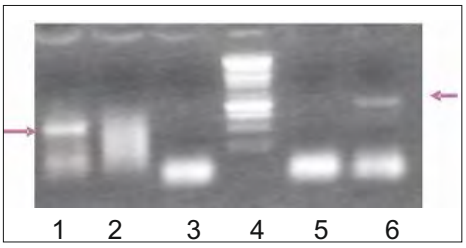


Fig. 5: Results of first PCR with Hantavirus with positive controls and sample

1. Positive control with Glycoprotein gene first PCR (560 bp),
2. Human serum sample with Glycoprotein gene first PCR (560 bp)
3. Negative control for RT-PCR of Glycoprotein gene,
4. 1 kb DNA ladder (Promega)
5. Negative Control for Nucleocapsid gene, first PCR (1051bp)
6. Positive Control for Nucleocapsid gene, first PCR (1051bp)

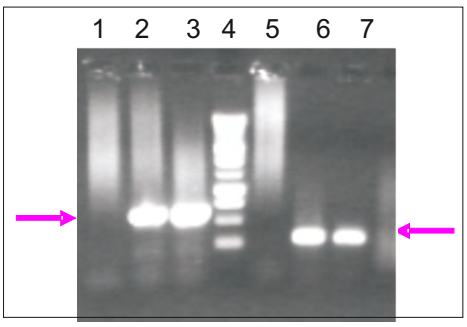


Fig. 6: Results of Hantavirus nested PCR with positive controls and sample.

1. Negative Control for Nucleocapsid gene, Nested PCR (599 bp)
2. Positive Control for Nucleocapsid gene, Nested PCR (599 bp)
3. Positive Control for Nucleocapsid gene, Nested PCR (599 bp)
4. 1 Kb DNA marker (Promega)
5. Negative Control for Glycoprotein gene, Nested PCR (317 bp)
6. Positive control with Glycoprotein gene first PCR (317 bp)
7. Positive control with Glycoprotein gene first PCR (317 bp)

ELISA

A rapid and sensitive ELISA based test to detect IgM antibodies to pathogenic human hantaviruses is ongoing

Molecular characterization of Bunyaviruses isolated from India

PD Yadav, PV Barde , Sandeep Kumar and DT Mourya

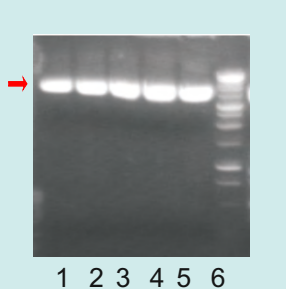
Objective

Molecular characterization of some suspected Bunyaviruses avaialble with NIV repository.

Work done

Molecular characterization of Ingwavuma virus (ING)

Two strains of ING virus (Pig strain no. 86208 & human strain no. 86627) were amplified by a single step gradient RT-PCR for the M gene and sequence analysis of the amplicon is ongoing.

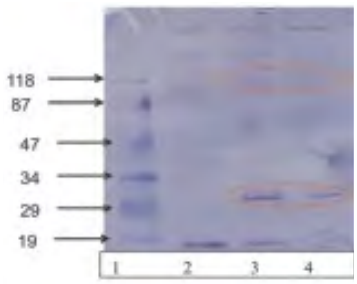


- 86208 strain amplification with bunya genus specific M gene primers at different temperature: {lane 1 - 5)
- 1kb DNA ladder (Promega): (lane 6) 86627 strain

Fig. 7: M gene amplification ING virus by a single step RT-PCR

Ingwavuma (ING) virus susceptibility in mosquito and one day old chickens

Culex quinquefasciatus mosquitoes were infected by intra-thoracic route with the 86627 virus strain of Ingwavuma virus and the virus multiplication was determined using IFA. None of the mosquitoes showed replication of the virus till 10th PID. One-day-old chickens were infected with ING virus by intravenous route. Based on partial N and M gene specific primers RT-PCR was done on chicken blood (drawn at different PID after 2nd day onward). Results showed that chickens did not support the multiplication of virus. The virus strains 86209 and 86627 were grown in RD cell line, proteins were transferred to NC membrane and probed with 1:100 diluted anti INGV immune serum raised in mice. Goat anti mouse Abs HRP (Sigma) was used at 1:20000 dilution. Two bands were detected one of 108 kDa probably of G1 and other 26-28 kDa, which can be G2/ N protein respectively (Fig. 8). Confirmation of the glycosylation of these proteins would make it clear whether 26-28 kDa are G2 or N protein.



Lane 1: Mol wt marker, Lane 2: Normal RD cells, Lane 3: INGV (86627), Lane 4: INGV (86208).

Fig. 8**:** Western Blot of cell lysate from INGV infected cells

Molecular Characterization of Chittoor viruses

Based on molecular and serological studies Chittoor virus has been placed in Genus Bunyamwera, family Bunyaviridae. Three strains of Chittoor virus, which are 804986, isolated from *Anopheles subpictus* in 1957, 804992 isolated from *Culex tritaeniorhynchus* in 1980 while 804988 isolated from *Culex tritaeniorhynchus* mosquitoes from Vellore field station. Virus stocks were prepared in Swiss albino mice and further adapted to Vero E6 cell line. After getting CPE on 3rd PID, cells were harvested for study. The complete M gene of 4.2 kb were amplified using conserved Bunyamwera genus glycoprotein gene specific primer by a single step gradient RT-PCR (Fig 9). Sequencing of these products is in progress.

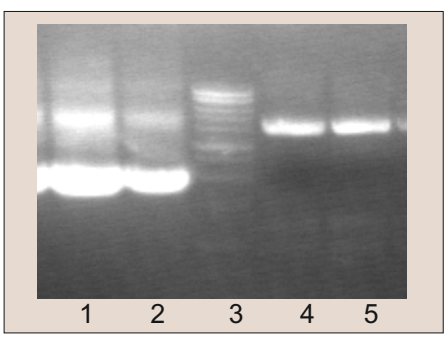
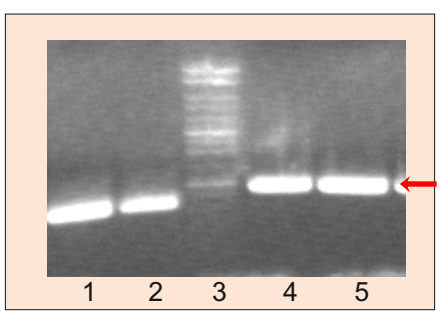


Fig. 9: M gene amplification of 804986 and 804992 Chittoor viruses by a single step RT-PCR:(Lane 1 & 2) 804986 strain amplification: (Lane 3) 1kb DNA ladder (Promega): (Lane 4 & 5): 804992 strain amplification.

1000 bp of Nucleocapsid (S) gene and partial 600 bp of RNA polymerase (L) gene were also amplified using Batai specific primers by single step RT-PCR (Fig. 10).



(Lane 1) 804986 strain amplification for partial L gene:
(Lane 2) 804992 strain amplification for partial L gene:
(Lane 3) 1kb DNA ladder (Promega):
(Lane 4) 804986 strain amplification for complete N gene:
(Lane 5) 804992 strain amplification for complete N gene.

Fig. 10: Complete N and partial L gene amplification of Chittoor virus by a single step RT-PCR

Studies on a densovirus isolate from mosquito

DT Mourya, MD Gokhale, PV Barde, Aruna Shivram and P. Yadav

Introduction

Densovirus (DNV) belongs to the family: Parvoviridae, subfamily: Densovirinae. The Densoviruses from mosquito *Ae. aegypti* and *Ae. albopictus* belong to genus Brevisenovirus. Densoviruses infect invertebrates and cause densonucleosis diseases. Their virions are small icosahedral, nonenveloped particles of 18-26 nm diameters. Their genomes consist of single stranded linear DNA molecules equimolecularly encapsidated as plus and minus strand. Their genome size ranges from 3.9 5.9 kb.

Objectives

Characterization of a densovirus isolated from mosquito

Possible role of densoviruses in interfering with CHIK in co-infected host mosquito

Work Done

Establishment of Densovirus infected mosquito colony

The *Aedes aegypti* mosquito colony was screened for DNV by PCR and some mosquitoes found positive. The infected ones were triturated and added to fresh water pans, the freshly hatched 1st instars larvae were added in this water. The emerging adults were again triturated and next generation was obtained similarly. After 5th generation individuals were tested by PCR, which were found positive for the DNV. The process of generation of highly infected colony in a continuous manner is being followed.

Preparation of immune sera

Immune sera was prepared in mice and IgG was purified . The serum was titrated against triturated virus *i*nfected *Ae. Aegypti* larvae found positive for DNV.

IgG purification and Dot Blot Assay

IgG fraction of immune serum was purified using Protein G column. Western Blotting was done to detect the structural proteins of virus. The ultra centrifuged purified pellet was re-suspended in GNTE buffer and immuno precipitation was done using purified IgG. The pellet obtained after immuno precipitation was used for western blotting to detect the viral structural proteins and IgG was used as the negative control. Antimouse IgG conjugated with Alkaline phosphatase was used for probing the blot. Duplex bands as reported for the viral proteins were observed in all samples except the IgG .

Electron microscopy

Transmission electron microscopic examination of the pellet by negative staining showed presence of a 27nm parvovirus-like particle at moderate frequency.

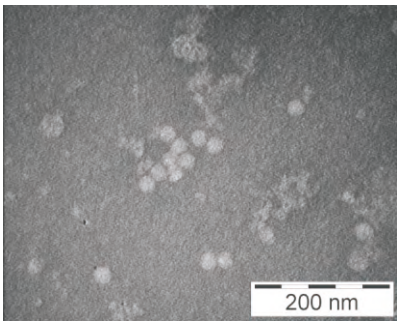


Fig. 11**:** Representative transmission electron micrograph of DNV virus in ultra-centrifuged pellet of infected larval suspension

Molecular characterization

The DNV was amplified using the virus specific forward and reverse primers, which gave a 1088 bp of products. Sequencing was performed using the amplification primers and two internal primers. Percent homology was 88% with *Aedes* densonucleosis virus (M37899.1), 74% with *Aedes albopictus* DNV (AY095135.2) and 48% with C6/36 cell densovirus (AY310877.1).

Establishment of method for decontamination of closed chambers in high containment laboratory

DT Mourya

Various conventional methods like gaseous, vapour and misting systems, fogging, manual spray and wipe techniques employing a number of chemical agents are used for decontamination of enclosed spaces. Formaldehyde is the most preferred method due to practicability and cost effectiveness in spite of irritating fumes, extremely corrosive nature and difficulty in maintaining a high level of gas concentration. The vaporization of hydrogen peroxide (H2O2) to ensure cent percent coverage of space volume and surface area with optimal concentration for achieving complete decontamination, poses certain practical difficulties. We have developed quick and reliable decontamination method, using plasma in combination with H2O2 (Table-2). Data suggests that with this combination quick decontamination can be achieved.

Table-2: Effect of plasma and H2O2 on the bacterial spores

Parameters	Bacterial growth in the media			
Distance from exposure source (cm)	Time of exposure min)			
	1	3	6	10
Plasma				
1	+	+	-	-
3	+	+	-	-
6	+	+	-	-
H_2O_2				
1	+	+	+	-
3	+	+	+	-
6	+	+	+	-
Plasma & H_2O_2 together				
1	+	-	-	-
3	+	-	-	-
6	+	-	-	-

Publications

1. Mourya DT, Yadav P. Vector biology of dengue & chikungunya viruses (Editorial). *Indian J. Med. Res* 2006, 124; 475-480
2. Mourya DT, Mishra AC. Chikungunya fever. *The Lancet* 2006; 368: 186-187

Participation in meetings, workshops

Dr. DT Mourya, DD

National Conference on "Role of Armed Forces in Disaster management", organized by College of Military Engineering, Faculty of NBC protection CME, Pune from 20-21 June 2006.

American Biological Safety Association annual conference at Boston, USA from 15 - 18 October 2006.

Invited speaker Emergence of Chikungunya, Department of Biotechnology, University of Pune on 27th Jan 2007.

One-day conference on "Good Laboratory Practices", organized by ISPE India, 30th March 2007 in Goa.

Meeting of the expert group for reviewing the progress of establishment of BSL-4 facility, MCC, Pune on 18th July 2006.

Conference on "Medical Preparedness & Mass Casualties Management" from 22-23 August 2006 organised by National Disaster Management Board, New Delhi.

International conference on "Biosecurity and International Nonproliferation Regimes" from 14-15 September 2006 organised by Institute for Peace & Conflict Studies, New Delhi.

Taskforce meeting on Chikungunya on 19th September 2006 organised by DBT, Delhi.

PD Yadav, RO

American Society of Virology conference from July 15th-19th 2006 at Wisconsin Madison, paper presented "Genomic comparison of Ganjam virus M segment with that of Crimean Congo Hemorrhagic Fever virus".

Lecture presented by Dr. Pierre Rollin, Special Pathogens Branch on 'Historical and Future Perspective on BSL4 Laboratories in the U.S' on February 1, 2006.

Weekly seminar series (24 meetings) highlighting current research on viruses in the Division of Viral and Rickettsial Disease (DVRD), CDC, Atlanta, USA.

"Applied Biosystem seminar on DNA sequencing fragment analysis and Real time PCR user group meeting, CDC, Atlanta, USA"

Online training in for “select agent' CDC from CDC/ATSDR office of health and preparedness .

Radiation training go through the online and practical training to use the Gamma cell irradiator training from CDC, Office of Health and Safety.

Select agent incident response training and tabletop exercise from Office of security and emergency preparedness .

Online training of Computer Security awareness from Department of Health and Human Services, CDC.

BSL-4 laboratory training - the video session and the walk through portion of the Bio safety level- 4 training provided by the Biosafety, Office of Heath and Safety, Centers for Disease Control and Prevention.

Presented a paper “Molecular Biology group”, SPB branch, journal club. “Bunyamwera Bunyavirus RNA Synthesis Requires Cooperation of 3'- and 5'-Terminal Sequences” CDC, Atlanta, USA.

Training program of DNA sequence analysis using GCG software in CDC during May 1-3, 2006, CDC, Atlanta, USA.

Presentation on “A single immunization with a recombinant canine adenovirus expressing the rabies virus G protein confers protective immunity against rabies in mice. Virol, 356 (2006) 147-154.” in NIV Journal club on February 23, 2007.

Participated in Hindi workshop organized on NIV, 15th Feb. 2007.

DR Patil, RO

Participated at seventh APCMV, Viral infectios in developing world 13th to 15th Nov 2006, New Delhi, India.

PV Barde, RA

American Society of Virology conference from July 15th to 19th 2006 at Wisconsin Madison.

Entomology

"Previous page shows the image of a tick, H. Spinigera, Vector of KFD"

Entomology Group

Scientific staff

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Dr. MD Gokhale MSc. Phd.

Technical Staff

Mr. AV Jamgaonkar

Mrs. MS Mavale

Mr. RV Aher

Ecological studies on Chandipura virus in Warangal and Karimnagar districts, Andhra Pradesh

P.CKanojia

Introduction

Although conclusive epidemiological evidence over the last few years have proved the association of CHPV with human encephalitis, the natural transmission cycle of this virus in nature remains incompletely understood. The virus could be detected earlier in the field collected sandflies by PCR. The present study focused on elucidation of the possible natural ecobiology of CHPV.

Objectives

To determine the species composition, population dynamics and host preference of sandflies

To detect and isolate CHPV from wild caught sandflies.

To determine the vertebrate host of CHPV

Findings

Species composition and population dynamics of sandflies in Warangal district

A total of 595 sandflies specimens representing 2 genera and 7 species namely, *Phlebotomus argentipes*, *Ph.papatasi, Segentomyia bailyi*, Sr. babu, *Sr. punjabensis* and *Sr.indica* were collected from their resting places. Two species namely *Ph. argentipes* and *Sr. bailyi* were collected throughout the year with maximum occurrence between January and June.Combined population density also showed little elevation from January to June and decline from July to December.Results of the study indicate that sandfly fauna of Warangal is not very rich in terms of species biodiversity. No significant elevation in population density was observed with regard to any species.

Table : Species composition and monthly incidence of sandfly species in Warangal district during 2006

Month	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Total
Species													
Ph. argentipes	47	38	32	08	03	10	08	01	03	15	43	14	222
Ph. papatasi	-	-	01	-	-	-	-	-	-	-	05	02	08
Ser. bailyi	42	27	27	16	36	20	20	10	06	13	09	03	229
Ser. babu	-	02	03	04	04	01	02	03	-	-	-	-	19
Ser. indica	-	01	-	-	-	-	-	-	-	-	-	-	01
Ser. punjabensis	01	02	03	20	14	27	15	06	12	07	07	02	116
Total	90	70	66	48	57	58	45	20	21	35	64	21	595

Sand fly species collected at Karimnagar district

A total of 81 sandfly specimens belonging to 2 genera namely *Phlebotomus* and *Seregentomyia* were collected from Karimnagar district form January to July 2006 with maximum occurrence in February.

Host preference of sandflies

One hundred seventy seven blood smears obtained from field caught sandflies namely *Ph. argentipes*, *Ph. papatasi, Sr. bailyi* and *Sr. punjabensis* were analyzed by precipitin test against human, cow, buffalo, sheep and pig antisera. *Ph. argentipes* was found positive for cow (36%), human (19%), pig (5.6%), sheep (3.3%) and buffalo (22%). Ph. papatasi showed host preference towards cow (28.8%), human (24.6%), pig (8.2%) and buffalo (8.2%).*Sr. bailyi* was also found positive for humans ,cow, buffalo, sheep and pig antisera.

CHP virus detection

As many as 183 field collected sandfly pools (re-pooled in 67) were processed for detection of CHPV by mosquito inoculation and immunofluoresence technique. Only 2 pools from *Sergentomyia* species were found positive (weak). Further passages are being conducted.

Serology

One hundred seventy four sera samples were obtained from different vertebrates namely rodents, squirrels, frogs, wall lizards and garden lizards with a view to determine possible involvement of these animals in the natural cycle of CHPV. In all , 112 sera samples were obtained from. *Rattus rattus rufescens*, *Mus musculus* and *Suncus murinus*. 38 samples (40%) tested positive for CHP neutrilizing antibodies. Only two samples (5%) from frogs were found positive for N antibodies. Similarly, 11% sera samples obtained from wall lizards showed presence of N antibodies. However, sera samples obtained from *Rattus norvagicus*, squirrels and garden lizards were found negative.

Entomological studies on JE at Warangal district, Andhra Pradesh (2006)

P.C Kanojia

Mosquito vectors and their seasonality

Seasonal prevalence and relative abundance of mosquito species, particularly JE vectors was determined in Warangal district. In all, 17845 female specimens representing 23 species in 5 genera were collected at dusk hours around animal

habitations.*Cx.tritaeniorhynchus*, the major vector of JE in India showed dominance over other species (62%) and was recorded round the year with elevated density from January to March. It showed single peak occurrence in February (114.6 PMHD). Whereas, this species presented bimodal pattern of peak occurrence during 2005 (115 PMHD in February and 188 PMHD in September). Warangal district received heavy rainfall and consequently suffered severe floods during 2006.This situation might have dislodged larval population of *Cx.tritaeniorhynchus*. And as a result, its population came down drastically during September (31.6 PMHD). Heavy downpour and floods also affected abundance of *An.subpictus* (secondary vector). It showed low density during July (14.4 PMHD) instead of high as observed in 2005 (99.4 PMHD). Abundance of other vector species such as *Cx.gelidus, Cx.pseudovishnu*i and *Cx.vishnui* was recorded extremely low during the course of study.

Mosquito species resting in animal habitations (indoors)

A total of 545 mosquito specimens comprising 10 species were collected during daytime from cattle sheds between August to December 2006. *An. subpictus* was found to be predominant species and shared large proportion of abundance (83.4%). Its PMHD (per man hour density) was recorded 13.3 individuals. *Cx. vishnu*i complex, including *Cx.tritaeniorhynchus* and *Cx. vishnui* together formed 7.3 % of the total collection.

Mosquito species resting in human dwellings (indoors)

A total of 5371 mosquito specimens representing 16 species were collected indoors during broad daylight period. *An.subpictus* was recorded in larger numbers (75%) and its overall per man hour density (PMHD) was recorded 17 individuals with highest occurrence in February (60 PMHD). *Cx. quinquefasciatus*, an arthophophilic mosquito stood 2nd with regard to percent prevalence (14%) and PMHD(3.1%) with no significant elevation in the population density from January to December. Remaining 14 species could not add much to the total collection.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Species													
An.annularis	-	01	-	-	01	-	-	-	-	-	-	-	02
An.barbirostris	01	-	-	-	-	-	-	-	-	-	-	-	01
An.culicifacies	03	-	01	-	-	-	01	-	-	01	-	-	06
An.peditaeniatus	01	-	-	04	-	-	-	-	-	-	-	-	05
An. stephensi	-	-	01	03	-	03	-	01	-	-	-	-	08
An.subpictus	560	1692	246	15	07	152	432	366	434	74	31	14	4023
An.vagus	41	34	16	02	-	-	-	-	-	-	-	-	93
Ae.aegypti	04	04	05	-	03	06	03	26	03	03	01	02	60
Ae.albopictus	-	01	-	-	-	-	-	-	-	-	-	-	01
Ar.subalbatus	25	13	76	37	20	04	03	16	08	-	04	10	216
Cx.(L)fuscanus	-	02	-	-	-	-	-	-	-	-	-	-	02
Cx.pseudovishnui	-	05	-	-	-	-	-	-	-	-	-	-	05
Cx.quinquefasciatus	43	95	100	93	73	87	93	103	09	13	29	11	749
Cx.tritaeniorhynchus	46	50	17	39	03	01	07	-	07	-	04	05	179
Cx.vishnui	01	10	-	-	-	-	05	01	02	-	01	-	20
Ma.uniformis	-	-	-	-	-	-	-	-	-	-	01	-	01
Total	725	1907	462	193	107	253	544	513	463	91	71	42	5371

Mosquito species breeding in paddy fields

There were 13 mosquito species (n=3872) found breeding in paddy fields, including JE vectors namely *Cx.tritaeniorhynchus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. bitaeniorhynchus.* Larvae of *Cx.tritaeniorhynchus* were collected in larger numbers (n=2847) with highest yield from January to March (n=2052). However, during monsoon period i.e, from August to October its population density was recorded very low (n=299). Low density of *Cx.tritaeniorhynchus* during rainy season which usually coincides with paddy cultivation can be attribute to the severe floods reported in the district due to heavy downpour.

Table : Mosquito species collected from paddy fields at Warangal district during (2006.)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Species													
An.barbirostris	-	-	01	-	-	-	-	-	-	-	-	-	01
An.culicifacies	01	01	-	-	-	-	-	-	-	-	-	-	02
An.pallidus	-	-	-	-	01	05	-	-	-	-	-	-	06
An. peditaeniatus	08	20	-	06	-	-	-	-	07	-	06	-	47
An. subpictus / An.vagus	93	208	62	01	06	88	08	33	-	-	-	-	499
Ar. subalbatus	01	-	-	-	-	-	-	-	-	-	-	-	01
Cx.bitaeniorhynchus	-	-	-	-	-	-	-	-	-	-	03	-	03
Cx.(L)fuscanus	53	03	-	01	-	-	-	-	-	-	09	-	66
Cx.pseudovishnui	-	11	82	-	11	18	14	04	-	-	-	-	140
Cx.quinquefasciatus	-	01	-	-	-	-	-	-	-	-	-	-	01
Cx.sitiens	03	-	-	-	-	-	-	-	-	-	-	-	03
Cx.tritaeniorhynchus	543	963	546	107	37	141	106	51	188	60	105	-	2847
Cx.vishnui	10	21	02	-	03	13	06	169	26	-	06	-	256
Total	712	1228	693	115	58	265	134	257	221	60	129	-	3872

Apart from paddy fields, mosquito larvae were collected from variety of artificial and natural breeding habitats. A total of 1990 immatures belonging to 12 species were captured. *Cx.tritaeniorhynchus* ,*Cx. vishnui*, *Cx. bitaeniorhynchus* and *Cx.vishnui* were found breeding in ponds and river basins. *Ae. vittatus* (n=1386) selected rock pools as preferred breeding places (n=988), however, its larvae were also collected from cement tanks, mud pots, plastic drums, grinding stone cavities, old tyres and metal pots. It was the only spices observed utilizing variety of artificial water containers for lying the eggs.

Table : Mosquito larvae collected from different breeding habitats other than paddy fields at Warangal district (2006)

Species	Cement tanks	Mud pots	Plastic drums	Grin-ding stones	Wells	Rock pools	Tyres	Metal pots	Ponds	River basins	Total
An. sub./vagus	--	01	-	-	-	-	-	-	05	-	06
An.culicificies	-	-	-	-	-	-	-	-	-	15	15
An.stephensi	42	02	-	-	16	03	-	-	-	-	63
Ar.subalbatus	-	10	-	-	-	-	-	-	-	-	10
Ae. aegypti	12	72	30	-	-	-	-	26	-	-	140
Ae.albopictus	-	71	-	-	-	-	-	-	-	-	71
Ae. vittatus	178	91	14	86	-	988	13	16	-	-	1386
Cx. bitaeniorhynchus	-	-	-	-	-	-	-	-	-	109	109
Cx. fuscitarsis	08	-	-	-	-	-	-	-	-	-	08
Cx. quinquifascitus	28	77	-	-	12	-	-	-	-	-	117
Cx.tritaenionrhynchus	-	-	-	-	-	-	-	-	-	45	45
Cx.vishnui	-	-	-	-	-	-	-	-	14	06	20
Total	268	324	44	86	28	991	13	42	19	175	1990

Monitoring of mosquito population using UV light traps

Two JE affected villages namely Lyabarthy and Pallaghutta were selected for monitoring mosquito population using UV light traps. A total of 10415 mosquitoes, including 11 JE vectors were captured in 61 overnight trap collections wherein, *Cx.tritaeniorhynchus* was captured in larger numbers (n=9211) followed by An. subpictus (n=716), An. vagus (n= 179)and *Cx. quinquefasciatus* (n=99) *Aedeomiya catasticta,* the vector of Alfuy virus (flavivirus) in Australia and Papua New Guniea was collected only in light traps.

Host selection of *Cx. tritaeniorhynchus* (major vector of JE)

One hundred fourteen blood smears prepared from wild caught *Cx.tritaeniorhynchus* were tested against human, cow, buffalo, sheep, pig, fowl and human plus bovine antisera by pecipitin test. Smears were found positive for all aforementioned antisera except human + bovine with maximum positivity to human, cow and buffalo antisera

JE virus detection

Attempts were made to detect JE antigen from variety of field caught mosquito by using mosquito inoculation technique. Thirty pools comprising 20 species and 949 individuals were processed, however, no virus could be detected from any pools.

Geographic genetic variation among the natural populations of Cx. tritaenionrhynchus and susceptibility to JEV

P.C. Kanojia, D. N. Deobagkar, and M. D. Gokhale

In India, *Cx. tritaenionrhynchus* is the principal vector of JE and widely distributed in endemic and non-endemic areas having dissimilar ecological conditions. This species is well known for its exophilic resting behavior. But it was found resting

indoors in huge numbers in Bellary district. Different geographic strains of *Cx. tritaenionrhynchus* have not been compared with regard to their morphological variations, biochemical analysis, variations at molecular levels and virus susceptibility status. *Cx.tritaenionrhynchus* strains collected from different states will be studied for above mentioned aspects to determine the differences at various levels.

Objectives

To determine the morphologic, iso-enzymatic and genetic variations among the natural populations of *Cx. tritaenionrhynchus.*

Differential susceptibility to JE among *Cx. tritaenionrhynchus* populations from different geological locations in India.

Work done

Cx. tritaenionrhynchus strain, brought from Bellary district (JE endemic area), Karnataka has been colonized successfully.

4th instar larvae from F1 generation have been examined for morphological variations.

Measurement of morphological characters of 4th instar larvae of *Cx. tritaeniorhynchus*

Egg rafts have been preserved for morphological examinations.

Bellary strains have been preserved for iso-enzyme and molecular study.

Bellary strain is being examined for virus susceptibility status.

Bangalore Field Station

Bangalore Field Station

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Activities of the Bangalore Field Station

Investigations on Flavivirus infections in Karnataka

PN Yergolkar and Hanumaih

Introduction

Clinico-epidemiological studies and surveillance of Flaviviruses of public health importance (viz,, DEN, WN) in areas of Karnataka

Objectives

Laboratory detection of JE, WN and DEN viral infections in suspected cases.

To study the epidemiological features and develop a disease data-base for these viruses.

Work done

Studies on JE in Karnataka State.

JE cases were diagnosed in 3 out of 18 suspected cases of encephalitis tested. Positive cases were children aged between 6 to 7 years in the month of October from Gulbarga district

Studies on dengue (DEN) in Karnataka State and neighboring districts of Kerala.

Out of 287 cases tested 36 (12.5%) were positive for dengue. Positive cases of dengue were from 13 districts of Karnataka in the months of October (13), November (7), January (3), February (2) and March (11).None of the 36 suspected chikungunya cases tested from Alappuzha district, Kerala state in the month of October were positive for dengue.

Entomological studies on *Aedes aegypti (Linnaeus)* in Karnataka

P George 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, Tamil Nadu and Kerala States between April 2006 to March 2007 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.

Work done

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 (Table 1).

Table 1: *Aedes aegypti* indices in CHIK outbreak localities.

State	No. of Districts Surveyed	No. of Villages/Towns Surveyed	Breteau Index (Range)	Adult House Index (Range)
Karnataka	7	30	40-200	20-70
Kerala*	2	6	62-70	24
Tamil Nadu	2	3	48-56	24-25

* *Aedes aegypti* and *Aedes albopictus*

Entomological studies in Tamil Nadu state

Epidemic suspected to be due to CHIK virus infection was reported from many districts of Tamil Nadu since June 2006. Entomological studies were carried out in Salem town of Salem district, Binamangala and Jawalageri in Krishnagiri district. *Aedes aegypti* was detected in all these three localities. The BI ranged from 48.0 to 56.0. Cement water storage tanks were the main breeding source.During the survey a total of 106 *Aedes aegypti* adults consisting of 27 males and 79 females were collected in indoor adult collection from these localities.Virus isolations attempts were made in 12 pools of 106 wild-caught *Aedes aegypti* adults (27 males in 4 pools and 79 females in 8 pools) and all these pools were processed for virus isolation in C6/36 cell line and 3 CHIKV isolates were obtained were from 3 pools of male mosquitoes collected at Bandari Nathan street on 16-6-06 in Salem Town and district of Tamil Nadu, 2 pools were with 10 males each and 1 was with 2 male mosquitoes. Earlier CHIKV was also isolated from one pool with 1 male mosquito collected on 24-1-06 at Hajnal village in Bidar district of Karnataka. CHIKvirus isolations were confirmed by IFA, RT-PCR and sequencing assays.

Entomological Studies in Kerala state

A major epidemic involving several thousands of cases suspected to be due to CHIKV was reported from many districts of Kerala state since July 2006. The district Alappuzha was the worst affected followed by Thiruvanathapuram. Entomological studies were carried out in these districts. Vizhinjam village in Thiruvanathapuram district was found positive for *Aedes aegypti* breeding and BI recorded was 70.6. In Alappuzha district breeding of *Aedes aegypti* was

detected only in town wards of the municipal area where as *Aedes albopictus* was found to be prevalent in all the rural areas surveyed. The Aedes index recorded in the town was 62.2. Tyre dumps shows maximum breeding of *Aedes aegypti* in some areas. *Aedes* species breeding was found in leaf axils of Glossina plants, cuttings of bamboo, colacasia plants, tree holes etc. which hold rain water become potential Aedes breeding sources. Other mosquito species recorded were *Aedes albopictus, Malaya genurostris, Armigeres auriolineatus, Tripteroides aranoides* and *Uranotaena christophersi.* A total of 139 *Aedes aegypti* and 98 *Aedes albopictus* adults were collected from indoor/outdoors. Other mosquito species collected are *Aedes vittatus* (1), *Culex quinquefasciatus* (5), *Mansonia annulifera* (6) *and Mansonia uniformis* (2) from these areas.Virus isolations attempts were made in 139 wild-caught *Aedes aegypti* in 16 pools and 67 *Aedes albopictus* adults in 9 pools. In addition 35 *Aedes aegypti* in 8 pools and 754 *Aedes albopictus* in 95 pools from larvae reared unfed adults were also processed for virus detection. All the pools were processed for virus isolation in C6/36 cell line and no virus isolates were obtained.

Entomological Studies in Karnataka state

Entomological studies were done in Jakkasandra village in Kanakapura taluk of Bangalore Rural district. The BI recorded was 68.0. During the survey 33 *Aedes aegypti* adults were collected. All the *Aedes aegypti* mosquito specimens were processed for virus detection in C6/36 cell lines. One isolate was obtained from a pool of 10 females collected on 26th February 2007. This isolate was confirmed as CHIKV by IFA.

Epidemiological studies on Chikungunya (CHIK) virus infections in Karnataka state and neighbouring states

PN Yergolkar

Introduction

Chikungunya virus outbreaks occurred after 32 years in India and for the first time in Karnataka State It was a large pandemic and the present study involved epidemiological aspects of the outbreak.

Objectives

To diagnose CHIK infections and outbreaks for prevention & control.

To study epidemiological features and molecular characterization of virus isolations from humans & vectors.

Work done

During the period from April to March 2007 a total of 5265 specimens were received from Karnataka (4861), Kerala (271), Tamil Nadu (120) and Andhra

Pradesh (13) states. A total of 1023 samples were processed for virus isolation in C6/36 cell line and 273 CHIKV isolations were confirmed, 331 were negative and in 419 virus isolations could not be completed. CHIK IgM ELISA was performed on 360 specimens and 112 (31.1) were positive for anti CHIK IgM antibodies. CHIK was confirmed in 25 out of 27 districts in Karnataka during the period. State wise confirmed cases were Karnataka-254, Kerala-114, Tamil Nadu-15 and Andhra Pradesh-2. CHIK virus isolations were obtained in all months of the period from April 2006 to March 2007 in Karnataka indicating continuous CHIK activity (Table 2). The Chikungunya virus was isolated from CSF of a 8 years old female child a suspected case of encephalitis from Tolahalli village of Kudligi taluk in Bellary district. The CSF and serum were collected on 2nd post onset day. CHIK virus was isolated in C6/36 cell line from CSF and reisolation was also successful. Virus could not be isolated from acute serum. Both acute CSF and serum were negative for anti CHIK IgM antibodies. However seroconversion and IgM to CHIK virus was shown in convalescent blood sample collected from the case on the 60th post onset day. The other CSF isolate was from a 6 years male child from Saidapur village of Shahapur taluk in Gulbarga district with onset in October and clinical diagnosis of viral encephalitis. CHIKV was isolated from 7th post onset day CSF and the case was positive for anti Japanese Encephalitis (JE) IgM antibodies in both serum and CSF indicating dual infection of CHIK and JE.CHIKV was also isolated from 4 days old male infant from 2nd post onset day serum sample in the month of August with a clinical diagnosis of viral fever/Septicemia.

Table 2: Month wise Diagnosis of CHIK cases from Karnataka State Apr-2006 to Mar-2007.

Month	No. of Samples processed for virus isolation	No. Positive for CHIKV isolation	CHIK IgM		Total Positive for CHIK
			No. Tested	No. Pos.	
April - 2006	86	47	191	37	84
May	42	21	-	-	21
June	51	37	-	-	37
July	76	53	-	-	53
August	42	24	-	-	24
September	14	4	-	-	4
October	44	4	-	-	4
November	58	6	-	-	6
December	47	4	-	-	4
January - 2007	11	4	6	1	5
February	10	6	9	5	11
March	35	1	38	3	4
Total	516	211	244	46	257

Sequence analysis of dengue viruses isolated from Karnataka

PN Yergolkar, Sandeep Kumar D and Mourya DT

Introduction

Dengue virus actvity has been endemic in different parts of Karnataka. In continuation of the studies from earlier years, the phlyogenetic analysis of these local dengue virus isolates was carried out.

During the period serotyping of all 105 isolates were completed. Of these, 96 isolates were obtained during 1993-2001 and 5 isolates were obtained in 2004

and 4 in 2005. All isolates were screened by RT-PCR using dengue virus group specific primers between 134 - 645 nucleotides position and the C-PrM region of the genome. PCR product of 511 bp size was sequenced and blasted against non-redundant Data Bank, which gave results of sequences matching with DEN-1 in 18 (17.1%), DEN-2 in 63 (60%). Remaining 24 (22.9%) of the isolates were negative for DEN in RT-PCR with the universal dengue primers used for screening and by IFA against anti dengue polyclonal mouse hyper immune serum. They were also negative for CHIK and Ingwavuma viruses by RT-PCR and for CHIKV by IFA. DEN-2 virus circulated in all the years with virus isolation during 1993-2005 and DEN-1 also circulated in Karnataka in the years 1995, 1996, 1999 and 2000 (Table 1). The possible evolutionary relatedness of these strains are being analysed.

Table 1: Serotyping of dengue isolates from villages of Karnataka

Year	No. of Isolates	DEN-2	DEN-1	Negative
1993	13	11	00	02
1995	14	02	07	05
1996	11	09	01	01
1997	24	19	00	05
1998	08	02	00	06
1999	09	01	06	02
2000	14	08	04	02
2001	03	03	00	00
2004	05	05	00	00
2005	04	03	00	01
Total	105	63	18	24
Percentage		60%	17.1%	22.9%

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 of Poliomyelitis Programme

PN Yergolkar and Hanumaiah

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 in Karnataka State.. NIV Field Station, Bangalore is responsible for processing all the stool specimens from Karnataka State.

During the period from April 2006 to March 2007 a total of 1506 stool specimens from AFP cases were processed for virus isolation. AFP cases were from Karnataka (581) and 12 other states (185). No wild poliovirus was isolated from any of the samples process. Virological results of AFP cases are Poliovaccine-41, NPEV only-262 and Negative-463. The break up of 41 polio vaccines are P1-18, P2-4, P3-4, P1+P2-2, P1+P3-8, P2+P3-1 and P1+P2+P3-4. During the

period 15 contact specimens from 3 index AFP cases were processed for virus isolation of which 10 were from Karnataka and 5 were from West Bengal State. Virological results were Polio vaccine virus-2 (one each P1+P3 & P1+P2+P3), NPEV only-9 and Negative-4. No wild poliovirus was isolated from any of the contact specimens. AFP surveillance indicator targets were fully achieved for the year.

WHO Accreditation for NIV Field Station, Bangalore in the WHO-SEAR Polio Laboratory Network

PN Yergolkar and Hanumaiah

Polio Laboratory was fully accredited by the WHO for the year 2006-07. Criteria and targets achieved during 2006 and are presented as following:

Target	Achievement
Tests are performed on at least 150 Stool specimens annually	1535 AFP cases stool specimen tested.
Score on annual onsite review is at least 80%	Onsite review Score is 99%.
Test results on 80% of all AFP specimens are reported within 28 days	100% - 1535/1535 reported within 28 days.
Accuracy of poliovirus typing is at least 90%	100% - 76/76 polio isolates confirmed.
At least 80% of poliovirus isolates from AFP cases are forwarded for intratypic differentiation within 7 days	100% - 76/76 polio isolates sent within 7 days
Results on most recent Proficiency Test Panel is at least 80%	100% for March 2007 Proficiency Test
Internal Quality Control procedures are implemented at least Quarterly	Implemented Internal Quality Control procedures Quarterly

Summary

No wild poliovirus was isolated during the period with high quality surveillance standards achieved in Karnataka state and it is more than 3 years without wild poliovirus isolation and the last wild P1 was isolated in February 2004 and P3 wild virus was isolated in December 2000.

Surveillance of Measles cases from Karnataka State, as a part of WHO-SEAR Measles Laboratory Network in the WHO's Global Measles Elimination Programme.

PN Yergolkar, 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. Laboratory diagnosis is carried out by detection of anti Measles and Rubella IgM antibodies using Dade Behring Measles IgM ELISA Kits. All Measles negative samples are tested for anti Rubella IgM antibodies. 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 & 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.

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 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 investigated and monthly report.

iTo 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 & Rubella by IgM ELISA

During the period from June 2006 to March 2007, a total of 365 samples were received from 19 district of Karnataka. Of these 254 (69.6 %) were positive for Anti Measles IgM antibodies and 111 were negative. Of the 111 Measles negative samples tested for Anti Rubella IgM antibodies, 20(18.0%) were positive and 91 were negative (Table 5). A total of 75 outbreaks were investigated in these 19 districts and 62 (82.7%) were positive for Measles, 5 (6.7%) were positive for Rubella. 4 (5.3%) were positive for Rubella and 4 (5.3%) were negative for both (Table 6).

B) Measles & Rubella virus isolation:

A total of 81 urine samples were received and processed from 17 districts for virus isolation in Vero/SLAM cell line and 10 Measles virus isolates were obtained from 7 districts with typical Measles virus syncitial CPE and 55 samples were negative. Chemicon IFA confirmed all 10 isolates as Measles. Genotyping was done in 9 isolates of which 6 were D8 and 3 were D4. Genotype D8 circulated in Koppal, Bijapur, Mysore and Gadag, D4 in Dakshina Kannada and Gulbarga districts, both D8 and D4 circulated in Kolar district. Measles outbreak was confirmed by IgM serology from outbreaks with measles virus isolations. Identification for Rubella virus isolation if any is to be done by IFA as it does not produce visible CPE.

Table 5: District wise Diagnosis of Measles cases from Karnataka State, Jun-2006 to Mar-2007.

District	Measles IgM			Rubella IgM		
	Tested	Pos.	Neg.	Tested	Pos.	Neg.
Bagalkot	15	10	5	5	-	5
Belgaum	5	4	1	1	-	1
Bellary	40	24	16	16	-	16
Bidar	15	14	1	1	-	1
Bijapur	5	5	-	-	-	-
Chitradurga	20	5	15	15	7	8
Dakshina Kannada	5	5	-	-	-	-
Davanagere	13	5	8	8	1	7
Dharwad	15	11	4	4	2	2
Gadag	5	4	1	1	-	1
Gulbarga	61	48	13	13	1	12
Haveri	5	5	-	-	-	-
Kodagu	4	4	-	-	-	-
Kolar	23	12	11	11	4	7
Koppal	32	30	2	2	-	2
Mandya	4	-	4	4	-	4
Mysore	10	9	1	1	-	1
Raichur	78	56	22	22	3	19
Tumkur	10	3	7	7	2	5
Total	**365**	**254 (69.6%)**	**111**	**111**	**20 (18.0%)**	**91**

Table 6: District wise Diagnosis of Measles Outbreaks Investigated from Karnataka State, Jun-2006 to Mar-2007.

District	No. of Outbreaks Investigated	Outbreaks Pos. for Measles	Outbreaks Pos. for Rubella	Outbreaks Pos. for Measles & Rubella	Negative
Bagalkot	3	3	-	-	-
Belgaum	1	1	-	-	-
Bellary	7	6	-	-	1
Bidar	3	3	-	-	-
Bijapur	1	1	-	-	-
Chitradurga	4	1	2	-	1
Dakshina Kannada	1	1	-	-	-
Davanagere	2	1	1	-	-
Dharwad	3	2	-	1	-
Gadag	1	1	-	-	-
Gulbarga	14	13	1	-	-
Haveri	1	1	-	-	-
Kodagu	1	1	-	-	-
Kolar	5	2	1	1	1
Koppal	7	7	-	-	-
Mandya	1	-	-	-	1
Mysore	2	2	-	-	-
Raichur	16	15	-	1	-
Tumkur	2	1	-	1	-
Total Outbreaks Investigated	**75**	**62 (82.7%)**	**5 (6.7%)**	**4 (5.3%)**	**4 (5.3%)**

Miscellaneous

Suspected Exanthem/HFMD like cases

PN Yergolkar, Hanumaiah, V Gopalkrishna and Chitambar SD

Investigation of suspected hand foot and mouth disease/Exanthem cases admitted to Indira Gandhi Institute of Child Health, Bangalore.

During the period from Dec-2006 to March-2007, several cases (about 50 cases) with clinical presentation of high grade fever of 1-2 weeks duration, erythematous maculopapular rashes all over the body involving palms and soles, with convulsions, altered sensorium, decerebrate posturing, some cases developing gangrenous changes of fingers, toes and ear lobes, vasculitis rashes similar to purpura fulminans and hepatomegaly were investigated for viral aetiology. Lab investigations indicated leucocytosis and thrombocytopenia. Mortality of about 10% and sequelae in 10% were observed with good recovery in others.

Cases were reported sporadically from 6 districts of Karnataka and 1 district of Andhra Pradesh in both male and female children of 1 to 10 years.

Blood, CSF stool, Throat swab and urine samples were collected from the hospitalized cases. CSF, Stool and Throat swabs from 18 cases were processed in RD, HEP-2 and Vero E6 cell lines for virus isolation. Serum & CSF were also processed in C6/36 cell line for virus isolation.

Serum samples were negative for WN, DEN, CHIK and Chandipura IgM antibodies and no virus could be isolated in C6/36 cell line.

Virus isolations (13) were obtained from 8 cases, 10 from stool and 3 from CSF. One of the stool isolate was obtained in all 3-cell lines. All isolates are being identified and aetiology of these cases is yet to be established, as all these isolates are negative for enteroviruses by RT-PCR

Publications

1 Mourya DT, Yadav P. Vector biology of dengue & chikungunya viruses (Editorial). *Indian J. Med. Res* 2006, 124; 475-480

2 Mourya DT, Mishra AC. Chikungunya fever. *The Lancet* 2006; 368: 186-187

Participation in meetings, workshops

Yergolkar PN

First Meeting of Virologists from WHO-SEAR Measles Laboratory on 5th Jun 2006

Member of Central Public Health Team to review/assess Chikungunya in Karnataka State. from17-19th Oct 2006

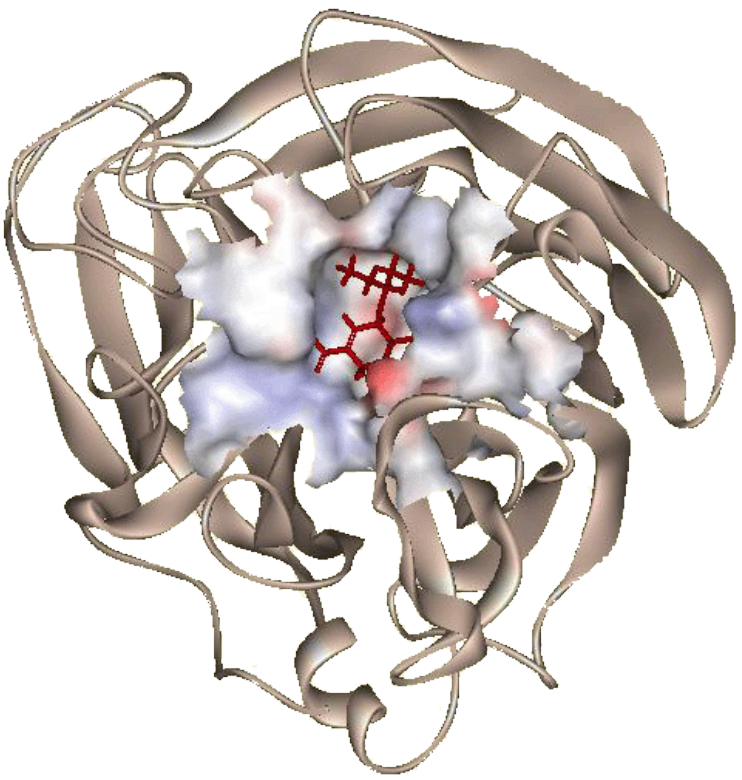
One Day International Dengue Meeting on10th Nov 2006 held at Pune.

15th Meeting of Virologists from WHO-SEAR Polio Laboratory Network on 4-5th Dec 2006 held at Haryana, India.

Polio Laboratory Coordinator's Meeting on22 - 23 Feb, 2007 at Trivendrum, Kerala.

Sir Dorabji Tata Symposium on Arthropod borne viral infections,. Invited Speaker, and presented paper on "Chikungunya outbreak in Karnataka and bordering states in Southern India, 2005-06. on 10 - 11 Mar 2007 held at I.I.Sc., Bangalore

Bioinformatics & Data Management



3D mode of H5N1 neuraminidase

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Molecular evolution of Chikungunya viruses

Sarah Cherian, A. Walimbe, S. Jadhav, V.A. Arankalle, A.C. Mishra

Introduction

The Indian ocean and Indian isolates of the 2005-2006 CHIK outbreak represented a clade within a broad group of East/South and Central Africa (ESCA). Isolates from the 2000 outbreak in Congo formed a separate clade within the ESCA group. A lone, whole genome isolate from Yawat, India also in 2000 did not match with the Congo group. Earlier isolates of the 1960's, 70's in India were of the Asian genotype. With this background, whole genome sequence analysis was carried out for recombination analysis and adaptive evolution. Further, structural studies were carried out to understand the divergence of the sequences isolated from the current outbreak in India with reference to the Yawat isolate.

Objectives

To carry out recombination analysis to check for inter- and intra-genotype recombination

To study adaptive evolution in the CHIK whole genome

Predict the tertiary structure of the E1 protein and map observed mutations

Achievements

No evidence for recombination could be observed from SimPlot and ML breakpoint analysis. Further, Maximum likelihood models using CODEML in the PAML software package (Yang, 1997) were applied to study adaptive evolution. No statistically significant evidence for positive selection was obtained (Table1). Towards structural studies, the Envelope glycoprotein E1 from Semliki Forest Virus (63% seq. identity) was used as a template to build a homology model of the E1 of the Chikungunya sequence of the S27 strain and map the mutations in the '05-'06 isolates as well as the 1963 and 1973 isolates of the Asian genotype (Fig. 1).

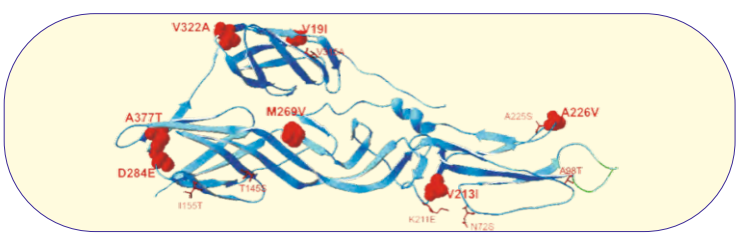


Fig1:Mapping of the mutations observed in the 2005-06 isolates (shown in spacefill and bold font) and those in Kolkata 1963 and / or Barsi 1973 (in wire frame and normal font.

Table 1: Likelihood values, parameter estimates and sites identified under positive selection under four models (M1a, M2a, M7 and M8) as applied to each gene.

Gene	*Model*	ln λ	% Sites under positive selection (ω ratio)	Positively selected sites *
Nsp1	M1a	-2782.90	-	
	M2a	-2782.28	0.5% (ω_2 = 8.27)	384 I, 507 L
	M7	-2782.96	-	**384 I, 507 L**
	M8	-2782.29	0.5% (ω = 8.26)	
Nsp2	M1a	-3969.05	-	
	M2a	-3968.59	4.8% (ω_2 = 10.92)	642 C
	M7	-3969.12	-	**642 C**
	M8	-3968.60	0.2% (ω = 10.90)	
Nsp3	M1a	-2928.03	-	
	M2a	-2928.03	6.2% (ω_2 =0.999)	337 T, 358 S, 376 I, 449 M
	M7	-2927.89	-	
	M8	-2927.89	2.6% (ω = 1.0)	337 T, **358 S**, 376 I, 449 M
Nsp4	M1a	-3207.92	-	
	M2a	-3207.92	1.8% (ω_2 =0.999)	43 A
	M7	-3207.89	-	**43 A**
	M8	-3207.89	0% (ω = 1.77)	
Capsid	M1a	-1323.29	-	
	M2a	-1322.90	5.6% (ω_2 = 1.88)	27 V, 63 K
	M7	-1323.45	-	**27 V,** 63 K
	M8	-1322.90	5.6% (ω = 1.88)	
E3	M1a	-335.19	-	
	M2a	-335.03	5.2% (ω_2 =3.2)	23 I
	M7	-335.27	-	**23 I**
	M8	-335.03	5.2% (ω = 3.2)	
E2	M1a	-2331.79	-	
	M2a	-2331.75	11.5% (ω_2 = 1.326)	57 G, 194 S, 211 I, 318 V, 377 I
	M7	-2331.80	-	
	M8	-2331.75	11.4% (ω = 1.329)	57 G, 194 S, 211 I, **318 V**, 377 I
6K	M1a	-301.70	-	
	M2a	-299.55	7.6% (ω_2 = 8.815)	8 V, **47 A**
	M7	-301.70	-	**8 V**, **47 A**
	M8	-299.55	7.6% (ω = 8.815)	
E1	M1a	-2291.45	-	
	M2a	-2291.45	1.6% (ω_2 =0.999)	211 K
	M7	-2291.43	-	**211 K**
	M8	-2291.43	0% (ω = 1.00)	

*** Sites identified with posterior probability > 50% of having ω >1 are shown. Bold font indicates posterior probability >75%.**

Conclusions

The isolates of the 2005-06 CHIK outbreak were not found to be a recombination of the African strains with the Asian strains. All the genes in the CHIK genome were under purifying selection with little evidence of positive selection. Future studies understanding the interaction of the E1 and E2 proteins and the implications of the observed mutations needs to be done.

Modeling the capsid protein VP6 of Avian Rotavirus, CH2 strain

Sarah Cherian, S. C. Chitambar,

Introduction

As indicated from sequence-based analysis of mammalian and avian rotavirus VP6 capsid protein, a stretch of very high variability was observed from res. 228 240 (9 out of 13 AAs different). This variable region may play a role in the expression of common antigenic epitopes shared by non avian rotaviruses. Based on the knowledge that the subgroup specific epitopes are conformational and present only in the trimeric form of VP6 (Lopez et al., 1994), a three dimensional structure of CH2 VP6 protein was predicted to emphasize and explain antigenic characteristics of the protein in relation to mammalian rotaviruses.

Objectives

To predict the structure of the avian VP6 CH2 strain.

Prediction of conformational epitopes of both mammalian and avian VP6 to determine the localization of group and subgroup specific residues.

Achievements

The structure of the avian VP6 CH2 strain was predicted using the Bovine rotavirus VP6 as a template. Molecular dynamics simulation of a hyper variable region (res. 228-240) was carried out by the simulated annealing approach followed by an overall energy minimization to predict the energetically favorable conformers (Fig. 2A). All simulations were carried out using the Discover module of Accelrys`s InsightII ver 2005. The sequential and conformational epitopes were mapped in the resulting structure using the Conformational Epitope Prediction (CEP) server (Kulkarni et al. 2005). Solvent accessibility of crucial regions was also determined by other methods.

Fig 2 (A): Superimposition of the template (RF strain) green and the modeled structure (CH2 strain) cyan in wire frame showing the hyper variable region (Res nos: 228-240) in the ribbon form.

Conclusions

The group epitopes are mainly linear while subgroup epitopes were found to be conformational as reported (Lopez et al., 1994). The critical predicted conformational epitope (CE) of the VP6 capsid protein of RF and CH2 strains (Fig. 2B) were multimeric. Amino acids A172, A305 and E315 that are known to be involved in defining the sub group epitopes of group A rotaviruses were found to be parts of this CE. The epitopes mapped on the distal domain of the 3D structure of the VP6 protein of both the strains reflect the antigenic diversity of group A rotaviruses of mammalian and avian species supporting earlier findings (Hoshino et al., 1984; Theil et al., 1989).

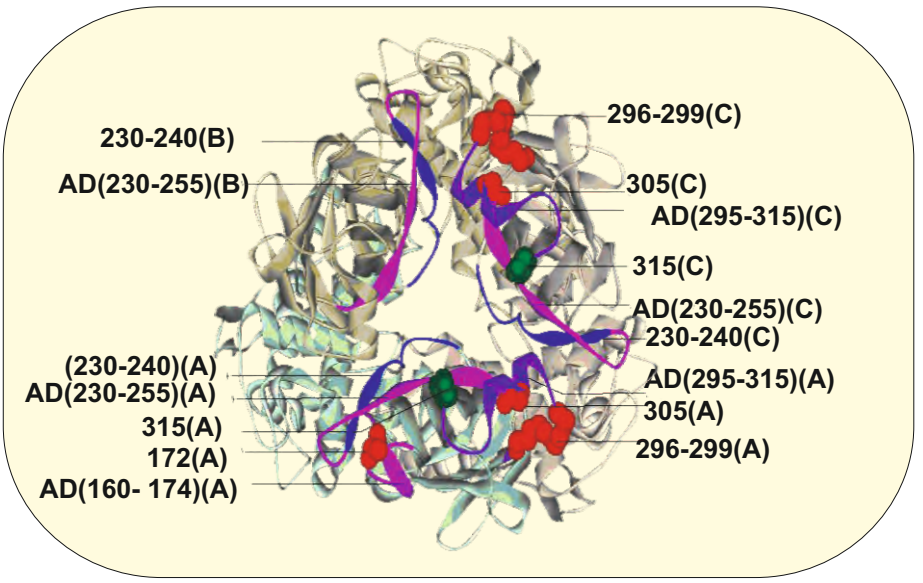


Fig. 2B: Axial view from the distal end of the trimer model of the CH2 strain. Predicted antigenic determinants and subgroup specific residues involved in the formation of a critical predicted conformational epitope is shown. The regions of group A epitopes (pink), SGI (red) and SGII (green) specific residues and the hyper-variable region (blue).

A-230-RLTQRAVIPTADGLNTWLFNPIILR-255-**A**
A-160-YNNSFTLIRSQPAHD-174-**A**
A-295-VRPPNMTPAVAALFPQAAPFP-315-**A**
B-230-RLTQRAVIPTADGLNTWLFNPIILR-255-**B**
C-295-VRPPNMTPAVAALFPQAAPFP-315-**C**
C-230-RLTQRAVIPTADGLNTWLFNPIILR-255-**C**

A Bioinformatics approach to study the interaction between dengue viruses and blood platelets

Sarah Cherian, Atanu Basu

Introduction

Earlier studies (NIV unpublished data) had suggested that exposure to dengue 2 virus leads to the loss of collagen agonist effect on blood platelets. In order to understand further the possible molecular basis of this interaction, the following study was planned using bioinformatics approach.

Objectives

Identify possible dengue 2 virus binding molecules on platelets and use of bioinformatics tools to understand molecular basis of dengue virus interaction with blood platelets.

Work Done

Preliminary modeling and homolgy search results suggests that the dengue viral E protein might have shared motifs for metalloproteinase binding with snake venom toxins which are also hemorrhagic. Futher studies are ongoing.

Core Activities

Bioinformatics

Studies in the area of genomic data analysis and molecular modeling of various viral proteins in collaboration with the experimental labs in the institute form the core activities. Sequence based approaches including phylogenetic analysis, recombination detection, selection pressure and molecular clock studies are applied for understanding viral molecular evolution. The structural models aid in explaining the virulent and non-virulent viral forms in terms of modifications in crucial properties such as antibody binding sites, conformational epitopes etc. Other advanced applications include docking studies for antigen-antibody interactions, design of immunogens and prediction of possible targets for the design of anti-virals/ inhibitors. The results of the studies help facilitate and validate wet-lab experimental studies, drug and vaccine design. The laboratory infrastructure has been upgraded to meet the advanced computational requirements.

Data Management

Data management pertaining to viral sample investigations is done through maintaining an SQL-based database which includes serological, entomological, and epidemiological data. Efforts are being made to integrate GIS (Geographic

Information System) data into it. Softwares for administrative data such as Pay roll system, purchase and inventory system, pensioner's application softwares have also been indigenously developed. Efforts are being made to convert the MS-Access database packages for administrative data into SQL-based systems.

Internet, LAN and Networking

The department administers two networks - one at NIV, Pune and the other at MCC, Pashan. Lease line connectivity between the Camp and Pashan offices has been established using a dedicated lease line of 2 mbps. Two CISCO 1841 series routers are used at both the ends for connecting the two networks. The LAN at NIV, Camp caters to 100 PCs and 12 workstations while that at MCC, Pashan to 80 PCs. At each location there is a windows 2003 server and an antivirus server. A 2 Mbps broadband Internet connection from BSNL at NIV, Pune, is distributed to MCC, Pashan, through the dedicated 2 mbps leased line.

Core Facility Services

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.

Development of software packages

In continuation with the development of IT application packages to help in the effective management of administrative and research data, the following has been done during the year.

Further improvisation and maintenance of the Pay-Bill package, stock room application, accounts application and Pensioners application package developed by converting into SQL database and integrating into an MIS towards achieving paperless office

Data entry, virus location package and viral database is under development.

Computer related services

Management of the LAN and internet services at NIV, Pune and MCC, Pashan.

A 2 Mbps leased line connectivity between MCC, Pashan and NIV, Pune has been established and the broadband bandwidth from BSNL has been increased from 1 Mbps to 2 Mbps at NIV. The leased line is being used to provide the internet connectivity to MCC, for intercom services and video conferencing.

Academics M.Sc. (Virology)

The section has been involved in conducting the following theory and practical courses for the Semester I and II of M.Sc. (Virology) program.

Biostatistics: Theory & practical course content development. Conducting lectures, practicals and the exams for the course.

Bioinformatics: Theory (1 credit) lectures & practical Course (1 credit)

Epidemiological data management: Practical course (1 credit)

Medical Entomology: Practical course 2 sessions

Guidance to project students of M.Sc. & M.Tech. (Bioinformatics), M.Sc. (Virology)

Publications

1 Genetic divergence of Chikungunya viruses in India (1963-2006) with special reference to the 2005-06 explosive epidemic (2007), Vidya. A. Arankalle, Shubham Shrivastava, Sarah Cherian, Rashmi S. Gunjikar, A.M. Walimbe, S. M Jadhav, Sudeep S, A.C. Mishra, J. Gen. Virology, 88, pp1967-76.

2 Chitamber S, Joshi M, Lole K, Walimbe AM, Vaidya S. Cocirculation of and coinfections with hepatitis A virus subgenotypes IIIA and B in patients from Pune, Western India. *Hepatol Res* 2007, 37 : 85-93.

3 Ray PG, Kelkar SD, Walimbe AM, Biniwale Vaishali, Mehendale Savita. Rotavirus immunoglobulin levels among Indian mothers of two socio-economic groups and occurrence of rotavirus infections among their infants up to six months. J Med Virol 2007 (March), 79(3): 341-349.

Participation in meetings, workshops

Dr Sarah Cherian

Asia-Pacific Congress on Medical Virology, 13-15 Nov. 2006, N. Delhi. Training on Applications of "Accelrys" Discovery Studio and "Accelrys" GCG package, 22- 28 Jan. 2007, National Institute for research in Reproductive health, Mumbai.

Mr Atul Walimbe

Asia-Pacific Congress on Medical Virology, 13-15 Nov. 2006, N. Delhi. Training on "Accelrys" Discovery Studio and GCG package, National Institute for research in Reproductive health, Mumbai, Jan. 22- 28, 2007.

Mr Vijayasimha

VBdotNet and SQL Server Course, APTECH, Pune, Jan - June 2007.

Mr Santosh Jadhav

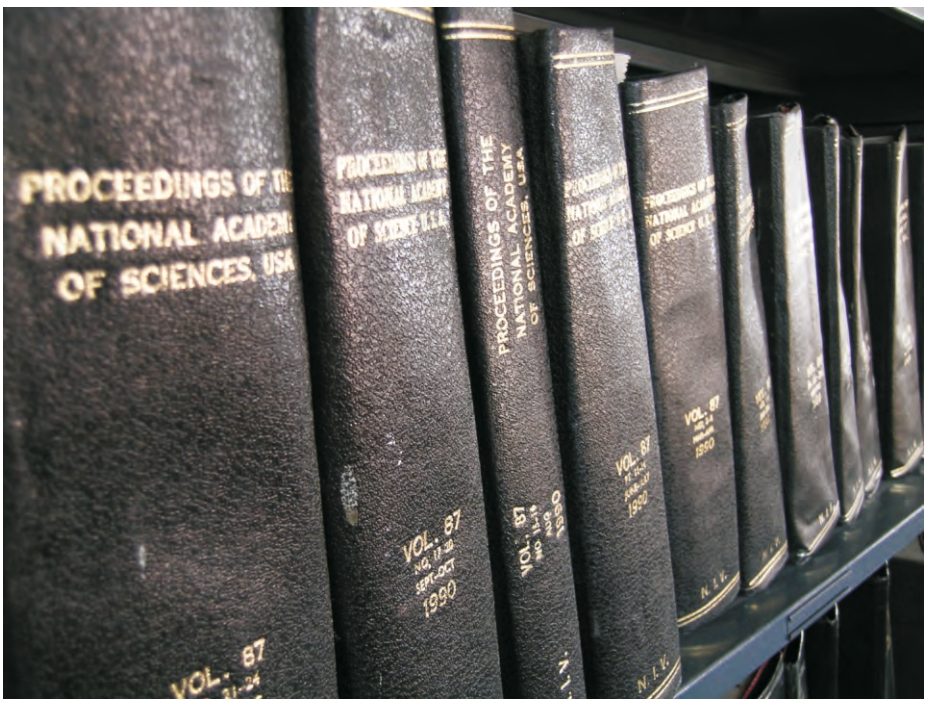
"Clinical trial, Diagnostic test and Cluster Design" and "Multiple linear regression, Logistic Regression & Survival Analysis" from May 22 - June 2, 2006 at CMC Vellore, Tamil Nadu.

"Knowledge Discovery in Life Sciences: Tools & Techniques in Bioinformatics" from January 29 - February 2, 2007 at Bioinformatics Centre, University of Pune, Maharashtra.

Core Facilities

CRYOTEM

CORE FACILITIES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OF SCIENCES, USA
VOL. 87
NO. 17-20
SEPT.-OCT.
1990


RESEARCH

Micoarray Facility

Scientific Staff
Dr. Mrs. Dhruba Ghosh, MSc. Phd., Group Leader
dhrubaa2010@gmail.com

The Microarray Facility is a recently created core research facility with the key objective of applying the tools of genomic research in the areas of basic virology and diagnostics applications. The major equipment are: GeneTAC Hybridization Station (Genomic solutions, USA), OmniGrid Accent Microarrayer (Gene machines, USA), SCAN-ARRAY Express Microarray Scanner (Perkin-Elmer USA). The major research activities of the facility falls into two groups (1) Core Facility Component (2) Development of Diagnostic arrays for viral infections.
The projects undertaken by the group are as as follows

- Development of oligonucleotide based assarys for influenza virus diagnostics
- Development of Amplicon based arrays using products generated from diagnostic PCR for the identification of viruses.
- Gene-expression profiling to study the differential response of host cells to encephatitis viruses in-vitro.

Electron Microscopy Core Facilty

Scientific Staff
Dr. Atanu Basu, Phd., Group Leader
basua@icmr.org.in
Techchinical Staff
Mrs. SV Gangothkar
Project Staff
Dr. Praksha Jain, Phd., (Research Associates)

The EM core provides high-resolution TEM application research base to researchers both within the institute and from other research facilities. The major thrust includes virus morphodiagnosis, immuno-EM, preparative techniques, cryo-EM applications and imaging. The core instrument clusters comprise of a 120 KV Cryo-TEM Tecnai 12 Biotwin (FEI Co, Netherlands) Transmission Electron Microscope (TEM) equipped with a ultra-high resolution Megaview III digital image acquisition module with an integrated I-TEM imaging platform capable of serial tomography reconstructions. The preparative cryo-platforms include a CPC universal station, Gatan 626 DH temperature controller with holder and a FCS rapid cryo-sectioning UM. As a part of continued quality assurance activity, two eternal quality assurance runs, EQA 16 and 17, were completed with the Robert Koch Institute, Berlin, Germany.

Ongoing Research Projects

Interaction of Dengue viruses with platelets

Preksha Jain, Shobha Gangodkar, Kanjaksha Ghosh *, Atanu Basu
Institute of Immunohematology, Parel Mumbai *
(Details given in Dengue Group Report)

Effect of dengue viruses on in-vitro hematopoiesis

Preksha Jain, Shobha Gangodkar, Kanjaksha Ghosh *, Atanu Basu
Institute of Immunohematology *
Extramural funding: Dept of Biotechnology, Govt of India, 2005-2008
Total: Rs 46.95 lakhs
(Details given in Dengue Group Report)

Characterization of the Dengue virus induced cytotoxic factor

Atanu Basu, UC Chaturvedi
(Details given in Dengue Group Report)

Ultrastructural characterization of small self-assembling peptides

Collaboration: International Center for Genetic Engineering and Biotechnology New Delhi

Summary

In continuation with earlier studies we continued with HRTEM analysis of synthetic dipeptides that showed presence of various self-forming structures ranging from nanotubes to large clusters. Further physicochemical characterization of these self-assembly process is undergoing.

Publications

Gupta M, Bagaria A, Mishra A, Mathur P, Basu A, Ramakumar S, Chauhan VS Self-Assembly of a Dipeptide-containing conformatinally Restricted Dehydrophenyl alanine Residue to form ordered nanotubes.Advanced Materials (2007) 19: (6) 858-861.

Animal House Facility

Scientific Staff
Dr. C.G. Raut, MVSc., Assistant Director & Group Leader
Dr. DR. Patil, MVSc., Research Officer
Technical Staff
Mr. BB Gengaje
Mr. SV Nipunage
Mr. HL Chakankar

Introduction

The laboratory animal facility at NIV is a state-of-art core facility that houses, breeds and cares for diverse animal species related to virological research. The most advanced animal maintaince systems including primate house is available to researchers. This facility works as an on-demand unit catering to all researchers. The provision for nude mice maintaiance and breeding is also present.

Summary highlights

The following species of animals are bred and/or maintained. The number of animals maintained ranged from 1832 to 2387.

Mice Swiss Albino, BALB/c, C3H/HeJ, C57BL/6, BL6 nude, NMRI nude, NIH nude, Golden/yellow Colour. Rats Wistar, Sprague Dawley, Charles Foster, Hamsters - Syrian Golden Guinea Pigs, Rabbits (NZW & Sandy Half Lop), Fowls, Geese, Sheep, Monkeys - Rhesus

A total of 3158 animals including 870 infant groups and 1376 adults of Swiss Albino mice, 25 infant groups and 594 adults of BALB/c mice, 88 C57BL/6 mice, 18 nude mice, 6 rats, 117 hamsters, 18 guinea pigs, 4 rabbits, 42 fowls were supplied to NIV scientists (Table-1 & Figure-1). 387 ml of Blood samples including 50 ml of Guinea Pigs, 180 ml of Rabbits, 52 ml of Fowls, 95 ml of Geese and 10 ml of Sheep were supplied to NIV scientists and outside institutions (Table-2 & Figure-2). A total of 3559 surplus animals including 2048 Swiss albino mice, 548 BALB/c mice, 647 athymic nude mice, 142 rats, 74 hamsters, 48 guinea pigs and 4 rabbits were supplied on request to various research organizations (Table-3 & Figure-3) registered with CPCSEA and a sum of Rs. 5,80,077/- has been collected towards the generated fund of the department.

Health Monitoring Programme

Assessment of athymic nude mice was carried out for T cell population at Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Navi Mumbai and at NIV, Pune.

Serum samples of various strains of mice & rats screened for murine pathogens viz. HVJ (Sendai virus), mouse hepatitis virus (MHV), Mycoplasma pulmonis & Tyzzer's organism. Animals maintained in conventional system were found reactive where as barrier maintained animals were found negative to mentioned pathogens.

Rhesus monkeys (20) screened for Herpesvirus Simiae/ herpes B virus antibodies by Dot blot ELISA, two monkeys found serologically reactive.

TB testing of monkeys carried out by employing tuberculin test, AFB (throat swab), Chest X-ray; all the monkeys were found non-reactive.

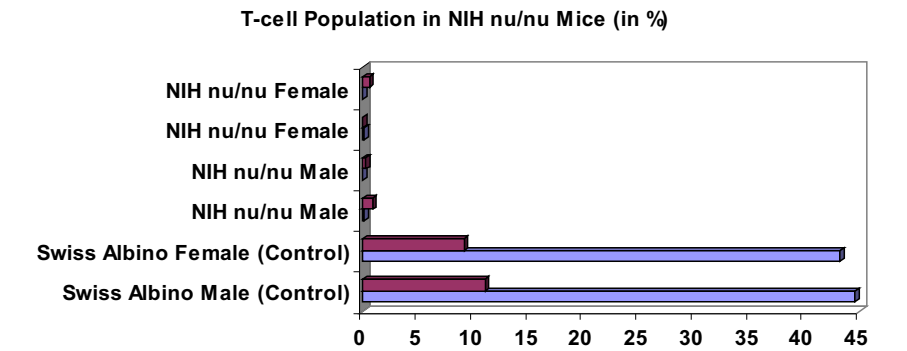
Old stored thirty serum samples of Rhesus (18), Bonnet (7) and Langur (5) monkeys were tested by DIA Dot ELISA (IgG Antibody) (Primates Products, USA) for Herpes B Virus (Herpes Virus Simiae). 5/5 Langur, 7/18 Rhesus and 2/7 Bonnet monkey samples were found reactive.

Quality Control Programme

A rigid environmental and dietary quality control programme for the animals is routinely carried out as per International guidelines and standards.

Assessment of T-cell population of Athymic Nude Mice

Assessment of T-cell population of NIH nu/nu males and females was carried out using Flowcytometry (FACS) technique. The results obtained indicate the athymic characteristics of NIH nude mice.



	Swiss Albino Male	Swiss Albino	NIH nu/nu Male	NIH nu/nu Male	NIH nu/nu Female	NIH nu/nu Female
CD8	11.07	9.17	0.87	0.2	0	0.59
CD4	44.63	43.25	0.06	0	0.1	0

Figure: T cells in nude mice

Table : Nude Mice Parameters

Parameters	*Diet (N)*	*Diet (G)*	*Diet (A)*
Number of young born	19	21	19
Average Litter size	8.5	10.5	9.5
Average Birth Weight	1.51	1.61	2.12
Total Males	11	9	9
Total Females	8	12	10
Male : Female ratio	1.4:1	0.75:1	0.90:1

Breeding performance of Athymic Nude Mice

Breeding performance of three different strains of athymic nude mice viz. NIH, NMRI and BL/6 maintained at NIV was studied for a period of five years (2002 to 2006). On an average 18 females of NIH strain, 24 of NMRI strain and 9 of BL/6 strain were used for breeding per annum. The total numbers of deliveries obtained per annum were 61 of NIH strain, 80 of NMRI strain and 32 of BL/6 strain. The average breeding performance per female per annum is given below

Table: Nude Mice Breeding

Parameter (per female per annum)	NIH Strain	NMRI Strain	BL/6 Strain
Average Deliveries	3.4	3.3	3.4
Young ones born	23.49	17.93	16.38
Litter size	6.91	5.43	4.82
Male : Female Ratio	1.71 : 1	1.89 : 1	0.78 : 1
Homozygous : Heterozygous Ratio	4 : 1	5.3 : 1	1 : 1
Homozygous Male : Homozygous Female	1.3 : 1	1 : 1	0.8 : 1
Heterozygous Male : Heterozygous Female	0.1 : 1	0.1 : 1	0.7 : 1

Staff Health Monitoring Programme

Health check up comprising Chest X-Ray, AFB in sputum, Haemogram, ECG, Blood Sugar (F & PP), Lipid Profile, physician checkup carried out at K.E.M. Hospital, Pune from 8.1.07 to 18.1.07. Most of the staff members showed the health parameters within normal limits.

Library Facility

Scientific Staff

Dr S.N.Singh, PhD, Library Information Officer & Head

Library Staff

Mrs. VV Yewale

Mr. HM Shinde

Ms. Meena Thakkar

Mrs. Vandana Kunjubihari

Mr. SK Deshpande

Mr. S Vishal Mali

Major Highlights

Proposal for new building for National Information Centre and Library for Viral Diseases was approved by the ICMR at MCC-NIV Campus, Pashan Pune. The Information Centre and Library of the institute has unique and rare collection of different varieties and various kinds of publications printed and digital 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. During the year 2006-07 the ICL added following documents and continued its services.

New Documents Added to Library

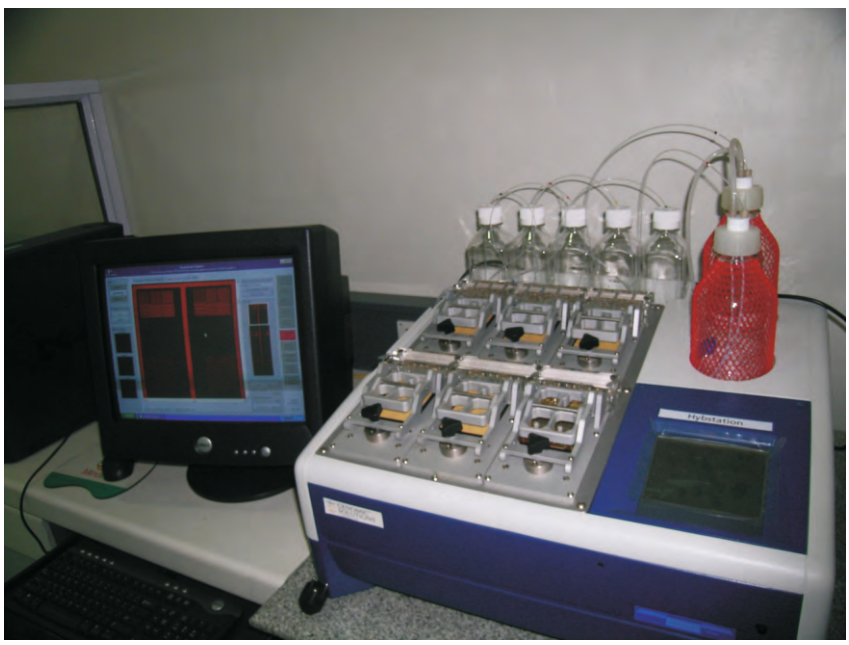
●	Books	-	681
●	Bound Volumes	-	71
●	Reports (miscellaneous) including Annual Reports	-	745
●	Reprints	-	743
●	Theses/dissertations	-	03
●	CD-ROMs	-	69
●	Current titles of the Journals:		
●	- Subscribed	-	72
●	- Gratis		158
●	Other documents	-	175

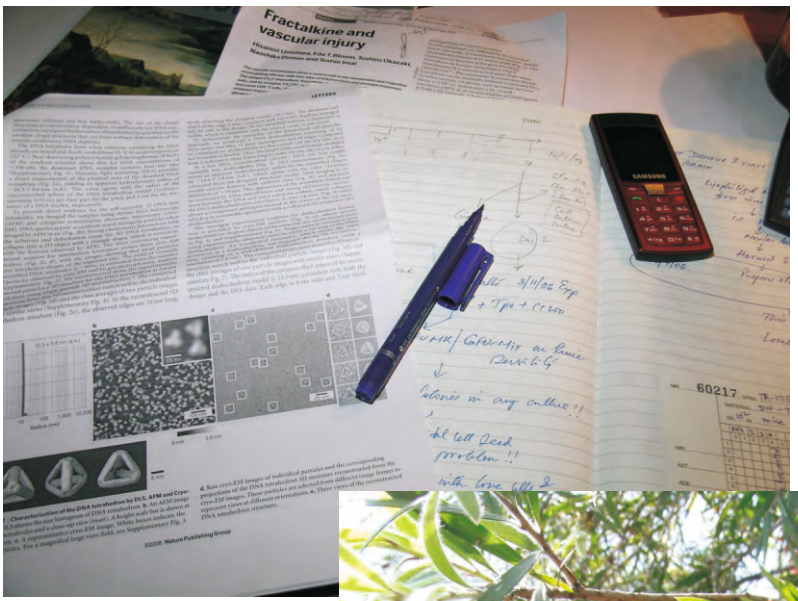
Services Provided

- Automation continued: Automated (first ICMR ICL) library-using LIBSYS and updated the documents as and when received as indicated above.
- On-line access of full text continued.
- Viral diseases database of Indian works discontinued due to lack of manpower.
- Libsys based OPAC service continued at NIV campus.
- E- journals, accessible against print versions, OVID, Pub med, Indmed, & free web based services provided and also necessary trainings given to access the above documents and databases.
- Users awareness of NIV-ICL was also provided to new comers including MSc Virology twice during the year.
- Started PhD Programme in Information and Library Science Topics (PhD Registered: 6; MPhil: 3)

.Publications

Singh SN. Communication technologies in biomedical information centers and libraries in India: A study. *Annals of Lib Inf Studies* 2006; 53(2): 70-73

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Administration

Administration

Mr. R Lakshminarayanan, Senior Admn Officer & Head Administration
rlnsudha@yahoo.com
Mr. SB Attar, Administrative Officer
Mrs. AV Varkey, Accounts Officer
Mr. VC Khunyakari, Sr. Stores Officer

Administration Staff

Mr. RB Vanarase
Mr. VV Pethe
Mr. SR Alle
Mr. SS Gole
Mr. SS Kale
Mr. SB Pandit
Mr. T Shankardasan
Mr. P Subramanian
Mr. UN Shetty
Miss. SN Ponkshe
Mr. N Shivsekhar
Mr. YK Mazire
Mr. NG S Nair
Mrs. SD Bhalerao
Mr. AS Gaikwad
Mr. JS Rangan
Mr. VP Pandharkar
Mr. DV Kulkarni
Mrs. PK Ratnaparkhi
Mr. DT Salunke
Mrs. AS Deshpande
Mrs. AS Palshikar
Mr. HS Pasalkar
Mrs. AS Bakare
Mrs. A Mathai Mr B K Wadke
Mrs. RK Amale
Mr. JR Kumbhare

The administration section of the Institute provides important support functions in various areas for streamline functioning of the institute. It develops and directs the implementation of policies and procedures and provides feedback to the Director/Group Leaders on problems encountered and matters requiring further attention which have department-wide implications. Provides guidance on the management of human resources and resolution of personnel issues like pay, recruitment and placement, training & development, employees' benefits, personnel transactions, timekeeping, payroll, leave, employees' performance evaluation, labor relations, promotions and retirement benefits etc. Oversee the budget and provides support services through: fiscal management, safety, security and environmental protection, project management, logistic support services, procurement and distribution management, Inter- and intra-office communications, conflict management etc.

LIST OF STAFF MEMBERS ATTENDED ISTM TRAINING DURING 2006-2007

Sr. No.	Name	Course Title	From	To
1	Mr. VR Jadhav, Section Officer	Establishment Rules	11.09.2006	15.09.2006
2	Mrs VV Shendye, UDC	Cash & Accounts	09.10.2006	08.12.2006
3	Mr. VC Khunyakari, SSO	Purchase Management in Govt	16.10.2006	18.10.2006
4	Mr. SE Matkar, UDC	Purchase Management in Govt	06.11.2006	08.11.2006
5	Mrs. RS Moghe, UDC	Purchase Management in Govt	06.11.2006	08.11.2006
6	Mr. SB Attar, Admn. Officer	Disciplinary Proceedings	13.11.2006	24.11.2006
7	Mr. VP Pandharkar, Assistant	Training programme on Reservation in Services	06.11.2006	09.11.2006
8	Mr. SR Alle, Section Officer	Training Programme on Financial Management in Govt.	20.11.2006	01.12.2006
9	Mr. R Lakshminarayanan, Sr. Admn. Officer	Course On Managing Change in Organization	27.11.2006	29.11.2006
10	Miss SN Ponkshe, Assistant	Course on Pension and Other Retirement Benefits	11.12.2006	15.12.2006
11	Mr. JS Rangan, Assistant	Establishment Rules	11.12.2006	15.12.2006
12	Mr. JR Kumbhare, Per. Assistant	40th Refresher Course	11.12.2006	22.12.2006
13	Mr. HS Pasalkar, Assistant	Assistants Refresher Course	18.12.2006	12.01.2007
14	Mr. N Shivshekhar, Assistant	Assistants Refresher Course.	18.12.2006	12.01.2007
15	Mr. NGS Nair, Assistant	Administrative Vigilance	05.03.2007	16.03.2007
16	Mrs. Shibi Jacob, Stonographer	41st Refresher course for Stenographers	12.03.2007	23.03.2007

MAINTENANCE

The services of Maintenance Staff were 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 530 works have been carried out major overhauling of central air-conditioning plant of animal house along with replacement of new cooling towers has been carried out.

Modifications to the new compressors of central air-conditioning plant of the old building, which could not be done by the supplier, have been carried out successfully.

Major overhauling to the cold room and freezer room machineries has been done to maintain optimum temperature out put.

Supports for high tech instruments and for equipments at Bangalore Field Unit have been provided.

Maintenance Staff

ST Perumal, Maintenance Officer & Head
YD Sable, Asst. Maintenance Officer (Sr.)

Technical Staff

VM Punekar
AR Sable
JM Mahadik
JD Pacharane

(6) Dr Shobha Broor
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(11) Dr S. Basu
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Scientific Advisory Committee

(1) Lt. Gen. D. Raghunath
Chairman
Principal Executive
Sir Dorabji Tata Centre for Research in Tropical Diseases
Innovation Centre
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(2) Dr G.C. Mishra
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(3) Dr T. Jacob John
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(5) Dr V. Ravi
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(12) Dr A.S. Kolaskar
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(13) The Director General
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(14) Dr D.A. Gadkari
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Institutional Ethics Committee

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(2) Mr. R.S. Soman, Member
43/9-A, Gumpha, Erndawane,
PUNE 41104

(3) Mrs. Sunanda Das, Member
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(4) Dr. S.M. Mehendale, Member
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(5) Dr. M.S. Chadha, Member
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(6) Dr. V.A. Arankalle, Directors Representative
Deputy Director, National Institute of Virology,
PUNE 411001

(7) Dr. A.S. Bhave, Member
Assciate Professor & Unit Head, Medicine Department,
BJ Medical Collage
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(8) R.V. Nangre, Member
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(9) Prof. R.K. Mutakar, Member
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Institutional Amimals Ethics Committee (IAEC)

(1) Dr. MG Deo
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(2) Dr. UV Wagh
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(3) Dr. Vikram S Ghole
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(4) Dr. GR Ghalsasi
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(6) Ms. Sunada Das
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(7) Dr. U Sengupta, Emeritus Medical Scientist,
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(8) Mr. Neelimkumar Khaire, Director, Indian Herpetological Society,
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(9) Dr. A. C. Mishra, Director, National Institute of Virology,
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(10) Dr. C G Raut, Senior Research Officer,
National Institute of Virology,
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Institutional Bio-safety Committee (IBSC)

(1) Dr. UV Wagh, Director, IRSHA, Pune / DBT Nominee

(2) Dr. AC Mishra, Officer-in-Charge, NIV, PUNE

(3) Dr. Bhaskar Saha, Scientist'E', National Centre for Cell Science, PUNE

(4) Dr. DT Mourya, Deputy Director, NIV, PUNE

(5) Dr. MS Chadha, Assistant Director, NIV, PUNE

Workshops/conferences/Seminrs/Training programmes / International meeting/symposium /conducted /organized

Workshop conducted

1. WHO-IVR "Onsite GCP and Device Training workshop for Clinical Investigators at NIV, Pune for Measles Aerosol Vaccine Project", on 3-5 April, 2006 at NIV, Pune
2. Organized the International Meeting on Dengue titled, "Overview of Dengue Epidemiology, Research Frontiers, Pathophysiology, Clinical Diagnosis, Treatment, Surveillance and Control." The meeting was held on 10th November 2006 at Hotel Le Meridian, Pune, hosted by Dengue Group, NIV, sponsored by ICMR and Pediatric Dengue Vaccine Initiative. Talks were by National and International speakers with about 200 registered participants from all over India.
3. WHO-NIV workshop on Review of Molecular surveillance network for measles in India, December, 2006, Pune

Training programmes conducted /offered

1. Hands-on training in IgM Capture ELISA to two staff members of CRME, Madurai from 30.10.2006 to 02.11.2006
2. Training offered to Dr Daw Lee Lee Lwin, a WHO fellow from Myanmar on laboratory diagnosis of JE/Dengue/Chikungunya.from 06- 17 November 2006
3. Two staff members of RMRC, Dibrugarh were trained for preparation of mouse brain antigen. from 20- 29 December 2006
4. Training/Workshop on "Influenza Virus Strain Surveillance" organized by National Institute of Virology, Pune. from January 20-24, 2007.
5. Training offered to staff of sentinel hospitals in Maharashtra, Gujarat and Goa on laboratory diagnosis of JE, Dengue and Chikungunya using kits developed at NIV, Pune. From 13-14 February 2007.

Visits organized.

1 Dr.Scott Haltead, PDVI visited the lab in 28 February 2006 and presented on "Antibodies determine virulence in dengue".
2. Visits from Dr.Jose Suaya, Brandies University (January 2006) and Dr. Dan Stichcomb & Dr. Jorge Osorio, Inviragen in June 2007.