# ANNUAL REPORT
## 2003-2004


**National Institute of Virology**
(Indian Council of Medical Research, New Delhi)

20-A, Dr.Ambedkar Road, PUNE - 411 001. India. Fax : 91-20-26122669
Tel. : 91-02-26127301 / 3, 26126302 / 4, E-mail : icmrniv@icmrniv.ren.nic.in


31st SCIENTIFIC ADVISORY COMMITTEE

**Late Dr. S N Ghosh memorial oration award to**
**Lt. Gen. (Dr) Raghunath from the Director, Dr. A C Mishara**


**A meeting on antiviral compounds**

# Preface

It is indeed a great pleasure to present the annual report of the National Institute of Virology, Pune for the year 2003-04. The year was very eventful. Satisfactory progress has been made in most of the priority areas and investigation of several outbreaks of unknown etiology was noteworthy.

We investigated outbreaks of encephalitis in children with high mortality in Andhra Pradesh, Maharashtra, Uttar Pradesh and West Bengal. Chandipura virus, a rhabdovirus discovered in 1965 by this institute was implicated as causative agent of the large encephalitis outbreaks in Andhra Pradesh and Maharashtra involving several hundred children from low socio-economic group. Influenza-A virus was found to be associated with the outbreak in Murshidabd, West Bengal. Despite extensive efforts, the causative agent for encephalitis outbreak in Saharanpur district could not be identified. This remains a challenge for our scientists. Both Chandipura and Influenza viruses were characterized at genomic level. Emergence of hepatitis A (genotype IIIA) in epidemic form in rural and semi-urban areas is cause of serious concern.

The progress reported for Japanese encephalitis, Dengue, Chikungunya, West Nile, Hepatitis, rota, respiratory viruses has been impressive. Some crucial products could not be taken to the logical conclusion due to the non-clearance of projects submitted to the large animal committee of the Government of India. This aspect is extremely frustrating because considerable investment has been made for the modernization of the animal house facility, which is also duly approved by the government.

Cordial relation and high level of commitment amongst staff members has been the haul mark for developing of good working environment. The clinical samples referred by state government were tested with top priority and very good interactive process was evolved to help the governments to manage the problem of emerging infections and the outbreaks. Large programs were organized and on-site training was imparted to health workers in Andhra Pradesh and Gujarat states.

We are grateful to Prof N. K. Ganguly, Director General, Indian Council of Medical Research, New Delhi for excellent support and encouragement. The guidance received from the Scientific Advisory Committee has been the driving force and we are grateful to all the members of the committee for their kind support and guidance.

We look forward for critical appraisals and suggestions.

A. C. Mishra
Director

# Mission

High quality applied and basic research in the areas of epidemiology, molecular biology, immunology, diagnostics, vaccinology, prevention and control strategies for viruses of public health importance, by creating a center of excellence, safe workplace and risk-free environment, through the establishment of state-of-the-art laboratories and development of appropriate human resources.

# Objective

- Studies on viral diseases affecting humans.
- Investigations of outbreaks, isolation and characterization of viruses.
- Providing diagnosis for viral diseases and development of indigenous diagnostic tests.
- Study of natural cycle, maintenance and spread of viruses.
- Developing models for prediction of viral epidemics.
- Developing animal models to study pathogenesis of viruses.
- Developing *in vitro* culture systems, including development of cell lines from mammals, arthropods and fishes.
- Studies on genetic and immunological properties of various viruses.
- Molecular epidemiology.
- Developing methods of prevention and control of viral diseases.
- Development and evaluation of vaccines and immunotherapeutics.
  Supply of reference virus strains and diagnostic reagents.
  Teaching and training of scientific and technical personnel.
- Creating awareness of viral diseases.



National Institute of Virology

Investgations/
Outbreaks

# CONTENTS



Annual Report 2003-04

# Viral Outbreaks Investigated by NIV

nivdo@vsnl.net

## 1. Investigation of outbreak of encephalitis in Andhra Pradesh.

### The Clinicoepidemiological features of outbreak

Cases of fever with CNS involvement (case definition by local authorities) started reporting from 1st June 2003. Over 329 cases and 183 deaths were reported till September 2003. Mainly, pediatric age group was involved, youngest being 5 months to 14 years. M: F ratio almost equal.

Typical clinical history of acute case was High grade fever of short duration followed by vomiting, generalized convulsions, altered sensorium, unconsciousness, decerebrate posture, leading to Grade IV coma and death within few hours of hospitalization.

There were no meningeal signs; pupils mildly dilated sluggishly reacting to light, bilateral papilledema in a few cases.

Few cases had loose motions as one of the symptoms but overall this was not the predominant feature. No cranial nerve involvement or neurological deficit was recorded. CNS type breathing and decerebrate postures were observed to be markers for bad prognosis. No respiratory symptoms in majority. Only one aspiration pneumonia examined. Brain stem infarction was detected on CT scans of few patients. Survivors were reported to have recovered without any deficit. Rashes were reported in few cases. Cases of encephalitis reported each year during this time but frequency is more than expected this year.

In Karimnagar, cases were spread out in 23 mandals of district Pedapalli mandal reported six cases with five deaths. Only one village reported 2 deaths otherwise clustering was not observed. Cases were observed to be on the periphery of the villages. Encephalitis spectrum was not reported in siblings of the affected cases. Though fever cases were reported in family. Only fever may represent milder spectrum for this episode. Animal deaths or any abnormal episodes (toxicity etc.) were not reported during this period.

The clinico-epidemiological features in Warangal were similar.

### Specimens collection

Eightynine specimens including blood, throat swabs, respiratory aspirates, CSF, urine, post-mortem brain aspirates, gastric secretions (aspirate), were collected from patients from Karimnagar and Warangal districts for virological studies.

### Laboratory investigations

Screening for endemic viruses viz. JE, WN, DEN, Measles, Paramyxoviruses, Coronaviruses, Enteroviruses, Influenza were negative.

Three of 22 throat swabs (first in MDCK cell culture; second in RD, Vero, MDCK; third in Vero, RD), one brain aspirate in RD and Vero cell cultures, and 2/10 blood clots in PBMC co-culture yielded virus isolates.

Electronmicroscopic examination of the tissue culture isolates showed presence of bullet-shaped particles, 150-165 nm long, 50-60 nm wide, showing distinct surface projections 9-11 nm in size and a stain filled canal at the base of the virus particles.





These isolates obtained from different cases were identified as Chandipura (CHP) virus by Complement Fixation and Neutralization Test.

CHP viral antigen and RNA were detected in brain tissue of a fatal case by IFA and PCR respectively.

A total of 4/21 throat swabs, 1/7 CSF, 5/25 sera, and one brain aspirate were found positive for CHP- RNA.

Sequencing studies revealed that these CHP virus isolates were identical (96-99.8%) with CHP reference strain isolated in 1965, thereby confirming the identity.

CHP IgM ELISA was developed indigenously at NIV within three weeks and serum samples were tested. 15/46 were found to be positive in encephalitis group. Also 2/10 sera were IgM positive in fever group and one contact indicating wide spectrum of the disease and possible sub clinical infections.

Neutralization tests and IgG ELISA also revealed presence of CHP antibodies confirming exposure to chandipura. One of 15 pools of Sandflies collected from affected areas was PCR positive for CHPindicating probable vector. Neutralizing antibodies were also detected in animal sera indicating exposure to CHP virus.

### Conclusion

Of the 55 encephalitis cases examined, evidence of recent CHP infection could be established conclusively in 28 cases (51%) based on either the presence of virus/ viral RNA and/or IgM antibodies. The failure to detect evidence of CHP infection in some samples could be due to the very early sample collection. A small proportion of the remaining cases perhaps represent encephalitis due to other causes. The multiple lines of evidence together implicates CHP virus as the etiologic agent associated with the present outbreak of acute encephalitis in the absence of any other identifiable causes.

## 2. Investigation of outbreak of encephalitis in Nanded district and some districts of Vidarbha regions of Maharashtra.

Outbreak of encephalitis in children was reported mainly from 9 districts of Maharashtra during June- August 2003. Clinical features included acute onset fever, headache, vomiting, altered sensorium, unconsciousness and death. Age group involved was mainly children below 10 years of age. Case fatality rate (CFR) was 41% from Nagpur region, highest from Bhandara (53%) and lowest from Nagpur (24%). The CFR was 30% from Nanded. In Majority of the cases, mortality was rapid, within 48 hours of hospitalization.

Encephalitis cases started appearing from 15th June 2003 and continued till end of August 2003. Total 476 encephalitis cases with 115 deaths were reported from Maharashtra. (Jan - Dec 2003- majority cases from June - August 2003). Cases were scattered in the region. Apparent familial clustering was not observed.

NIV teams visited Nagpur region and Nanded district and collected serum, CSF, brain

aspirates, throat swabs from cases, and serum samples from control group and domastic animals.

Laboratory investigations included IgM ELISA for JE, WN, Dengue and Measles in initial stages on suitable specimens. PCR for Paramyxoviruses, Influenza, Coronaviruses, Enteroviruses was also carried out. Immunoflurosence Assay was done on brain material obtained from an encephalitis case.

Initial results indicated that three specimens were positive for JE. Others were negative for JE, WN, and Dengue viruses. Since majority of the specimens were negative for the known endemic virus (JE) and CHP etiology was detected from Andhra Pradesh specimens during the same period, the specimens from this episode in Maharashtra were subjected to serological investigations of CHP etiology. CHP IgM ELISA was developed indigenously at NIV and standardized for use in the investigations.

CHP IgM ELISA was positive for 41of 202 (20 %) serum specimens from encephalitis cases, and 6 of 61 (10%) from fever cases and 14 of 103 (14%) from control/ contacts group. This indicated the spectrum of the disease, which needs further study. CHP IgG and Neutralisation Antibody assay was also performed on the serum samples collected. The initial results indicated positivity in cases and also positivity in control group indicating the background exposure to CHP virus. IFA performed on brain aspirate of fatal case of encephalitis from Nanded also proved CHP virus etiology.

**CHP IgM Serology status of encephalitis cases in Maharashtra during 2003**

| District | Reported Deaths/ Attack (CFR %) | Encephalitis CHP +ve/Total | Fever CHP +ve/Total | Others CHP +ve/Total |
|---|---|---|---|---|
| Amravati | 3/8 (38%) | 0/3 | 2/24 (8%) | 0/2 |
| Bhandhara | 16/30 (53%) | 6/31 (19%) | 0/0 | 1/ 4 (25%) |
| Chandrapur | 21/52 (40%) | 3/26 (12%) | 0/1 | 0/2 |
| Gadchiroli | 4/9 (44%) | 0/5 | 0/0 | 1/ 4 (25%) |
| Gondia | 6/14 (43%) | 3/12 (25%) | 1/ 2 (50%) | 0/1 |
| Nagpur | 29/123 (24%) | 11/67 (16%) | 0/13 | 0/0 |
| Wardha | 9/29 (31%) | 10/35 (29%) | 1/21 (5%) | 0/2 |
| Yavatmal | 4/12 (33%) | 0/3 | 0/1 | 0/0 |
| Nanded | 13/43 (30%) | 8/20 (40%) | 0/0 | 10/88 (11%) |
| Rest | 10/156 (6%) | NR | NR | NR |
| Total | 105/320 (24%) | 41/202 (20%) | 4/62 (6%) | 14/103 (14%) |

Data of cases (Not samples) Multiple positives from one case = one positive
Others include contacts, controls and clinical information not available

**Age Sex distribution of CHP Encephalitis cases from Maharashtra**

| Age groups (in years) | Male CHP +ve/Total | Female CHP +ve/Total |
|---|---|---|
| < 1 | 0/0 | 0/1 |
| 1-5 | 7/51 (14*) | 5/41 (12*) |
| 6-10 | 11/46 (24) | 10/25(40) |
| 11-15 | 4/13 (24) | 3/14(21) |
| 16+ | 0/3 | 0/1 |
| NK | 1/5 | 0/5 |
| Total | 23/118 (19) | 18/87(21) |





## 3. Outbreak of encephalitis in children in Saharanpur district, U.P.

First case of Encephalitis this year was reported on 12th Sept 2003. Cases increased thereafter to 34 as on 20th Oct 2003. Case definition of Acute fever with altered sensorium of less than seven days was set for surveillance. All the cases from the district were admitted to the district hospital isolation ward.

### Clinical features

Acute fever of 3-4 days duration, vomiting, altered sensorium, convulsions in few cases, unconsciousness leading to death in majority of cases. The fever was moderate to high degree. Two cases had a history of vomiting as first symptom followed by fever and other symptoms mentioned as above. The age range of the cases was 2 - 9 years, which includes 15 Males and 19 Females.

The cases were scattered throughout the district with no apparent epidemiological linkage with each other. Baliakhedi (8) and Puwarnka (5) were the most affected blocks. Usually one case was reported from each village. There were no similar cases in the family or neighbourhood. No sibling affected. Only in one case (Afsa) unconfirmed history reported sibling (2 years old) as having kanthi-Khasra (measles like illness). No major illnesses were reported in the siblings.

Out of 34 cases as on 20th Oct, 2003, 27 deaths, 2 were referred to PGI, Chandigarh (one died as per family history), 2 left against medical advise (LAMA), 2 were under treatment and one case recovered.

The CFR was 80% according to Saharanpur health authorities. Referred and LAMA cases were in serious condition. If we consider mortality in referred and LAMA cases then the Case fatality rate amongst encephalitis appears to go up to 91%.

The only Recovered case was traced in the field. This case had history of Vomiting- 5 days, fever-4 days, irritability-1day with altered sensorium and was hospitalized. No neck rigidity was observed. Child was treated and discharged in two days after recovery. No neurological sequalae were observed. Blood sample from this case was collected.

There was no history of any major illness in the encephalitis cases preceding the present episode. Upper respiratory tract infection was observed in case villages but apparently no increase in number of such cases. Fever survey done by local authorities in the case villages did not reveal significant increase.

Few children were anemic with Hb- 6- 7 gm%. No rashes or hemorrhages were observed in the cases. Diarrhoea was not observed. No jaundice was observed. The cases were managed symptomatically with antibiotics, steroids, mannitol, oxygen, IV fluids etc. History of abnormal behaviour was given in two cases. Irritability was observed in few cases. Neck rigidity was observed in two cases.

NIV has collected specimens from acute cases, contacts, and recovered case. The

specimens include blood, CSF, throat swabs, brain aspirate, urine and rectal swab. Aliquots were also given to the NICD team including brain aspirate. Total 70 specimens including blood and CSF samples collected by local health authorities were brought to NIV for investigations. NIV laboratory investigations are in progress.

## Serology

### Mac ELISA for JE and WN

MAC ELISA was done on 27 serum and 21 CSF samples for JE/WN. A pair of Serum and CSF sample from one patient showed presence of IgM antibodies against Japanese Encephalitis.

### IgM ELISA for CHP

Twentyseven serum samples were tested for CHP antibodies. No specimens were found positive for CHP IgM antibodies.

### Measles IgM ELISA

Measles ELISA was done on 22 serum samples using Behring IgM ELISA kit supplied by WHO and 2 serum samples from two cases had IgM antibodies.

## Polymerase Chain Reaction (PCR)

PCR was done on 7 throat swabs for Paramyxoviruses and CHP and were found to be negative.

PCR was done on brain aspirate from encephalitis case for Paramyxoviruses/ enteroviruses / CHP/ Flaviviruses / JE and WN and was negative for all these viruses.

## Virus isolation

Virus isolation attempts are in progress using suckling mice, different cell lines and coculturing with human PBMCs.

## Nipah virus Serology

Four serum samples were sent to CDC, Atlanta, USA for Nipah virus serology and were found negative.

# 4. Investigation of outbreak of unknown fever in Murshidabad, West Bengal.

An outbreak of encephalitis in children below 10 years of age in Murshidabad district of West Bengal, an eastern state of India was reported during May-June, 2003. The affected children had high-grade fever, upper respiratory infection, cough, altered sensorium, vomiting, and convulsions. Death occurred within 48 hours in majority of the cases. Of the 211 fever cases reported by the State surveillance till June end, 61 (29%) had predominant features of encephalitis and 48 (22%) had pneumonia. A total 39 deaths were reported, with Case Fatality Ratio of 13.7%. Convulsions were noted to be marker for bad prognosis of the patient. No neurological deficits were detected in survivors.





Though majority of the cases were from pediatric group, fever cases in adult were also reported but the magnitude could not be ascertained. Encephalitis cases in adults were not reported by State surveillance. Fever survey in one of the affected village did not detect any adult encephalitis case. Malnourishment in children was perceived to be an important factor in fatal cases.

During the peak period of the outbreak, thirty throat swabs and 30 blood specimens were collected from 30 acute cases with fever, respiratory and neurological features in different combinations. (Table 1) These also included symptomatic contacts of death cases. Specimens were initially screened for endemic viruses, like JE, WN, DEN, Measles (by IgM ELISA) and Paramyxoviruses and Enteroviruses (by nested RT-PCR using consensus primers), which yielded negative results. Serum neutralization test for CHP virus was also negative.

Sixteen of 30 throat swabs were posiitive for Influenza A by RT-PCR using primers based on M region of the genome as described earlier. These were negative for Influenza B. Partial M gene sequence of PCR products (Genbank accession #. AY544208 tO AY544211) from this episode showed 99% identity with several H3N2 Influenza strains.

Subsequent virus isolation attempts using standard procedures with throat swabs in MDCK cell line yielded 4 isolates which were identified as Influenza A (H3N2) in Haemagglutination Inhibition (HI) test with reference fowl and sheep Influenza antisera from Centers for Diseases Control and Prevention (CDC) Atlanta, USA.

Partial H gene sequencing done on one of these Influenza A (H3N2) isolates showed 96 % identity with A/Zhejiang/8/2002 (H3N2) and A/Ningbo/17/2002 (H3N2) strains.

All the 15 PCR positive cases had fever, respiratory and neurological manifestations in different combinations. Five of these cases were symptomatic sibling contacts of death cases. PCR positivity and Influenza virus isolation from these cases was a surprise finding as so far influenza virus was not suspected as possible etiological agent causing such outbreaks in India.

# 5. Investigation of Measles outbreak at Nilwande dam colony, PHC Vitha, Sangamner Dist: Ahmadnagar.

An outbreak of measles was reported from a small labour colony (approximately 1000 population) situated at Nilwande Dam. This labor population belonged to different states such as Bihar, West Bengal, Karnataka and Maharashtra, mainly involved in construction work of Nilwande dam. NIV team visited the affected area with PHC staff on 8 Mar 2004. NIV investigated the episode from etiological point of view.

Clinical features of the cases included fever associated with maculopapular rash, cough, coryza and conjunctivitis. This outbreak started in third week of January 2004. As per record of PHC, in the month of January 14 cases were observed whereas 77 cases reported in February. Only 5 cases were reported in March. Index case was not traceable. Immunization

status in most of the cases was not known but majority were perceived to be unimmunised.

NIV team collected blood samples (74), throat swabs (3), urine (1) sample and skin swab (1) and transported to laboratory in ice-cold conditions for serological and virological studies. Total 26 blood samples collected from suspected measles cases, 22 blood samples collected from contacts including mothers, and 26 blood samples collected from unaffected control population. Complications involving pneumonia and diarrhoea were reported. Also two deaths (1yr M and 3yr F) were reported with major complication of pneumonia.

This outbreak was confirmed by detection of IgM ELISA for Measles (Behring Kit) and by RT-PCR and sequencing. Of the 26 serum samples from suspected measles cases, 11 (42.3%) were positive. These were mostly recovered cases. None from control (5) and contacts (14) tested were positive for IgM antibodies.

This confirms the outbreak to be of Measles. Genotype D8 sequences were obtained from throat swabs of 2 acute cases in this outbreak. Virus isolation attempts from two acute cases of Measles are in progress using B95a cell line.

## 6. Investigation of conjuctivitis epidemic

An epidemic of viral conjunctivitis was reported in many areas of Maharashtra and Gujarat state during September to November 2003. These outbreaks were investigated to determine the aetiological agent of conjunctivitis epidemic and characterization of the isolates by immunologic, molecular and other methods.

### Clinical Specimens

Eye swab and throat swab specimens collected in VTM and acute serum samples were received from Sassoon General Hospital Pune, K.E.M. Hospital Mumbai and P.S. Medical College & Shree Krishna Hospital Karamsad, Gujarat.

| Name of Hospital | Nature of clinical specimens | | | Total |
|---|---|---|---|---|
| | Eye swab | Serum | Throat swab | |
| S.G.H. Pune | 153 | 17 | - | 170 |
| K.E.M. Mumbai | 07 | - | 07 | 14 |
| P.S.M.C.& Shree Krishna Hospital Karamsad, Gujarat | 20 | 30 | - | 50 |

### Inoculation of specimens in cell cultures

Initially Vero, RD and HeLa cells grown in 24 well plates (Nunclon) were used for inoculation of eye swab and throat swab suspensions after filteration through 0.22 µ filters. HeLa cells were found most susceptible. Hence, most of the specimens were inoculated on the subconfluent monolayer of HeLa cells.

### Isolation of virus

Out of 127 specimens inoculated, 27 virus isolates (24 from eye swabs and 3 from throat





swabs) were obtained. Amongst these 20 were from Pune, 3 from Gujarat and 4 from Mumbai specimens. The virus isolates showed initiation of CPE after 48 Hrs of inoculation. The specimens negative for virus isolation were passaged thrice in HeLa cells and are now being inoculated in Hep-2 cells. Two isolates were obtained from eye swab specimens after inoculation in Hep-2 cells, which were earlier, found negative inr HeLa cells.

### Neutralization test

Initially one isolate each from Mumbai (039094) and Pune (038837) were tested with standard immune serum against Coxsackie A 24 (CA24) and Enterovirus 70 (EV70) viruses. Both virus isolates were neutralized by CA 24 immune serum while a little cross neutralization was observed with EV 70 immune serum.

Eight more isolates (5 from Pune and 3 from Gujarat) were tested against convalescent serum collected from the Mumbai patient from whom the virus (039094) was recovered, showed neutralization with CA 24 immune serum. All these isolates showed neutralization against the convalescent serum indicating that the isolates could be the strains of Coxsackie A 24.

### Mouse pathogenicity

The virus isolates (5 from Pune, 2 from Gujarat and 1 from Mumbai) passaged in HeLa cells were used for inoculation in 1-2 Day old infant mice by intraperitonial route. The sickness was observed from 6$^{th}$ or 7$^{th}$ post infection day (PID) and persisted for more than 4 weeks. The mice showed spastic and flaccid paralysis in fore limbs and / hind limbs. The mortality varied from 50-90%.

### Diagnosis by PCR

The tissue culture derived virus isolates were checked in RT PCR using Enterovirus specific common primers. Nine virus isolates tested showed positivity against Enterovirus. These were also checked positive when with CA 24 specific primers.

### Summary

During the epidemic of Conjunctivitis, clinical specimens viz. eye swabs, throat swabs and acute serum samples were received from Pune, Mumbai and Gujarat. Eye and throat swab specimens were inoculated on the subconfluent monolayers of HeLa cells. Twenty-seven virus isolates were obtained from the eye and throat swab specimens. Some of the isolates were identified by NT with standard serum of Coxsackie A24 virus and convalescent serum sample and showed neutralization. These were confirmed by RT-PCR with CA24 specific primers. Eight isolates showed pathogenicity to infant mice.

## 7. Investigation of outbreaks of Viral Hepatitis

An outbreak of viral hepatitis among children was investigated at village Tathawade, Pune Twelve cases of hepatitis occurred in early October. The age groups affected were children below 10 years of age. The village received intermittent piped water and leakages were found

Common source outbreak of Hepatitis A due to well water contaminations

in the water pipelines. None of the 10 children tested were IgM-anti HEV or HBsAg reactive, IgM-anti HAV was positive in all indicating HAV etiology of the outbreak

An outbreak of hepatitis occurred at the CRPF colony at Daund, Pune district. The outbreak started in the 3rd week of December 2003 and peaked in the first week of January 2004. Water supply of affected areas was derived from 2 wells and was not chlorinated during December and January. Across the road, water supply was from the municipal corporation and no case was observed in this population. A total 117 cases of hepatitis among children <15 years of age (total pediatric population of affected area = 1052) were recorded. All were positive for IgM-anti-HAV antibodies confirming that hepatitis A virus was the etiological agent. The age group affected most was 5-10 years (43 cases). The Male: Female ratio was 1.96: 1. 11/12 stool samples and 5/11 serum samples collected from different patients and 1/1 sewage sample screened for HAV RNA in nested RT-PCR were positive. All the PCR products were sequenced and based on the phylogenetic analysis were typed as genotype IIIA. A serological survey was conducted among children in the entire colony. At the end of the outbreak 22% and 29% children remained susceptible to hepatitis A in the affected and unaffected areas respectively. One hundred and seventytwo such children were vaccinated.

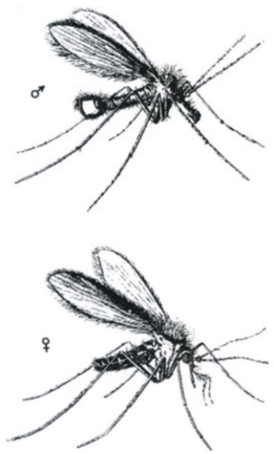
## Investigation of an outbreak of hepatitis A from Kerala

An outbreak of hepatitis A was detected in children from a school located in Nilambur, Kerala State during August 2003. The cases continued to occur up to February 2004. Pairs of serum stool were received from 18 children at NIV, Pune in March 2004. Eleven of the twelve children who suffered from hepatitis and three of the six children who remained unaffected during the outbreak showed positivity to anti-HAV IgM identifying HAV as an etiological agent of outbreak. HAV RNA positivity detected by RT-PCR in few stool/serum samples indicated faecal excretion and viraemia of HAV in children with subclinical/clinical hepatitis A.





# Chandipura Virus



Sandflies

## 1. Serological survey of domestic animals for antibodies to CHP virus.

**MV Joshi** mvjoshi46@rediffmail.com

An outbreak of acute encephalitis with high mortality among children in 11 districts of Andhra Pradesh was reported between June 2003 and September 2003. Over 329 cases with 183 deaths (Average case fatality rate 54.9%) were reported. Fever with convulsions or coma were the important symptoms. The aetiological agent of this epidemic was identified as CHP (CHP) virus. Maximum cases were reported from Karimnagar and Warangal district. As a part of the investigation of this epidemic, a serological survey of domestic animals (pig. goat, cattle, sheep and dog) was carried out in Karimnagar and Warangal district of Andhra Pradesh between 19 and 24th July 2003.

### Objective

Serological survey of domestic animals from Karimnagar and Warangal districts, Andhra Pradesh for prevalence of antibodies to CHP virus.

### Achievements

One hundred and eighty animal sera were collected from 5 villages in Karimnagar (92) and 7 villages in Warangal (88) districts of Andhra Pradesh. The distribution of samples collected from different species of animals is presented in the following table.

**Distribution of animal sera collected from different villages of Karimnagar and Warangal district of Andhra Pradesh, India**

| Villages | Pig | Goat | Cattle | Buffalo | Sheep | Dog |
|---|---|---|---|---|---|---|
| **Karimnagar district** | | | | | | |
| Munjampalli | 8 | 5 | 4 | 5 | 8 | 0 |
| Nijahitguda | 0 | 1 | 1 | 0 | 0 | 0 |
| Manakondur | 2 | 5 | 0 | 0 | 5 | 0 |
| Vety. Polyclinic | | | | | | |
| Karimnagar town | 0 | 3 | 0 | 4 | 0 | 0 |
| Kolanoor | 2 | 12 | 9 | 9 | 7 | 0 |
| **Sub total** | **12** | **26** | **14** | **18** | **20** | **2** |
| **Warangal district** | | | | | | |
| Kasibugga | 8 | 0 | 0 | 0 | 0 | 0 |
| Karimbad | 4 | 0 | 0 | 0 | 0 | 0 |
| L.B. Nagar | 13 | 0 | 0 | 0 | 0 | 0 |
| Warangal town | 0 | 8 | 6 | 6 | 1 | 4 |
| Pallagutta | 4 | 0 | 6 | 2 | 0 | 0 |
| Timmanapet | 2 | 7 | 2 | 2 | 5 | 0 |
| Tamadapalli | 6 | 2 | 0 | 0 | 0 | 0 |
| **Sub total** | **37** | **17** | **14** | **10** | **6** | **4** |
| **Grand total** | **49** | **43** | **28** | **28** | **26** | **6** |

All the animal survey sera were tested in 96 well plates by *in vitro* Neutralization test in Vero cell culture to detect antibodies to CHP virus. CHP immune serum prepared in Swiss albino mouse served as a positive control and the normal mouse serum was the negative control. Virus neutralizing (N) antibody titre was expressed as the reciprocal of the highest antibody dilution capable of neutralizing 100 $TCID_{50}$ of virus.

### Summary

Thirty-three of the 180 (18.3%) sera had N antibodies to CHP virus. Analysis of the sera according to the species indicated that 15 of 49 (33.6%) pigs; 4 of 43 (9.3%) goats; four of 28 (14.3%) cattles; five of 28 (17.9%) buffaloes and two of 26 (17.7%) sheep showed the presence of N antibodies to CHP virus. The highest seroprevalence was observed in pig followed by buffalo, cattle, goat and sheep. Three of the 6 dog sera were positive for N antibodies to CHP virus.

**Results of *in vitro* neutralization test on animal sera against CHP virus**

| Species | Andhra Pradesh | | No. positive/ No. tested | |
|---|---|---|---|---|
| | Karimnagar | Warangal | | |
| Pig | 2/12* | 13/37 | 15/49 | (30.6)** |
| Goat | 3/26 | 1/17 | 4/43 | (9.3) |
| Cattle | 2/14 | 2/14 | 4/28 | (14.3) |
| Buffalo | 2/18 | 3/10 | 5/28 | (17.9) |
| Sheep | 1/20 | 1/6 | 2/26 | ( 7.7) |
| Dog | 0/2 | 3/4 | 3/6 | (50) |
| Total | 10/92 | 23/88 | 33/180 | (18.3) |

* No positive/No. tested.
** Figures in parenthesis denote percentage.

### Future plan

It is planned to carry out viremia studies in domestic animals with CHP virus and also to study the immunological response and persistence of antibodies to CHP virus in experimentally infected domestic animals.

Sandfly collection from an anthill

## 2. Ecological and epidemiological studies on CHP virus in Warangal / Karimnagar districts of Andhra Pradesh.

**PC Kanojia** kanojia pc @ yahoo.co.in
**G Geevarggese, MV Joshi**

CHP virus activity has been detected in human / animal sera and sandflies collected during July and August 2003 from Karimnagar and Warangal districts of Andhra Pradesh. However, information on the natural cycle of CHPV is scanty. Hence these studies were undertaken.

### Objective

- To determine the species composition, population dynamics and host preference of sandfly and mosquito species prevailing in the district.
- To detect and isolate CHP virus from field collected specimen by using different methods.
- To determine the role of vertebrate hosts in the natural cycle of CHPV.

### Achievements

Indoor and outdoor collections of resting adult sandflies were made in human dwellings, cattlesheds and from treeholes during July 2003. A total of 185 sandflies belonging to *sergentomiya* sp.were collected in 7 villages of above districts. Of these, 15 pools, each consisting of two sandflies, were subjected to nested RT-PCR and one pool was found positive for CHP virus.

### Future plan

Intensive studies on the ecology of CHP virus are planned from July 2004 in the above districts.





## 3. Experimental infection of CHP in animals and its transmission through sandflies and mosquitoes.

**G Geevarggese** geeverghese_niv@yahoo.com
**AC Mishra, MS Mavale**

Chandipura (CHP) virus has been isolated/detected earlier from sandflies belonging to *Phelobotomus* and *Sergentomyia* species collected in Maharashtra and Andhra Pradesh respectively. In addition, experimental transmission has been achieved in six species of mosquitoes including *Ae. aegypti*. With a view of studying the vector potential of sandflies and mosquitoes species, transmission studies were undertaken under laboratory conditions.

### Objectives

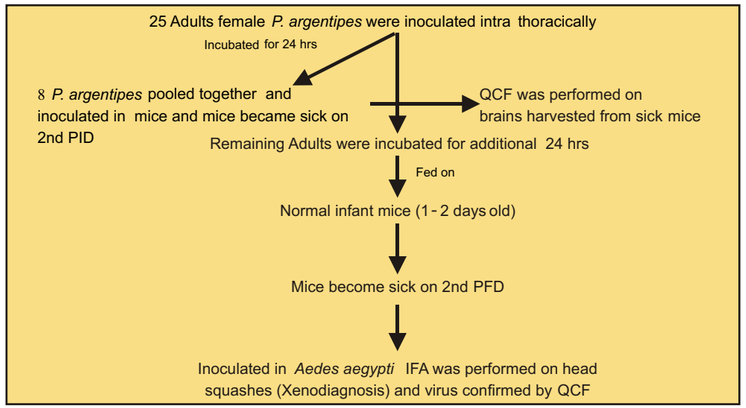
- To determine the vector competence of certain sandflies and mosquito species to CHP virus.
- To study the susceptibility of different animals to CHPV.

### Achievements

**1. Transmission of CHP virus by *Phlebotomus argentipes***

Laboratory reared *P. argentipes* sandflies (aprox. 25-30) were infected intrathoracically with CHP virus. After 24 hrs, these sandflies were fed on one-day-old infant mice. On 2nd post-feeding day (PFD) mouse became sick. Brain was harvested and sonicated in 0.75% BAPS, and centrifuged at 10,000 rpm for one hour at 4°C. Supernetant was collected and inoculated in normal *Ae. aegypti* mosquitoes. After 24 hrs incubation period mosquito head squashes were tested for the presences of CHP virus antigen by IFA and found positive. Quick complimentary fixation test was performed to confirm the CHP virus using mosquito bodies. Result indicated that *P. argentipes* sandflies could transmit virus to susceptible host. The experiment was replicated with identical results, indicating the transmission potential of *P. argentipes* to CHP virus by inoculation. However, attempts to infect *P. argentipes* by oral feeding were unsuccessful.

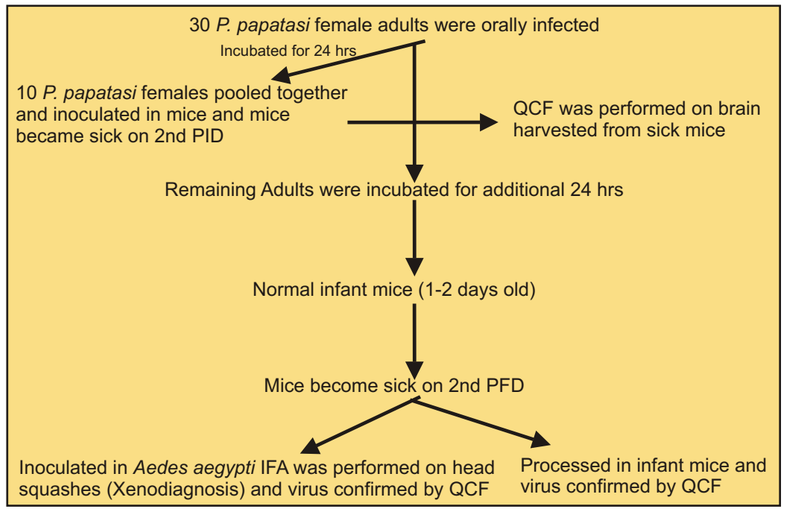
**Transmission of Chandipura virus by *Phlebotomus argentipes***



## 2. a. Susceptibility studies and transmission of CHP virus in *P. papatasi*

CHP virus was inoculated in 1-2 days old infant mice. On 1st PID 2-3 days old laboratory reared *P. papatasi* (approx.30-35) were fed on above viraemic mice. Feeding was observed during daytime. These sandflies were refed on 2nd day on normal 1-2 days old infant mice. The mice were sick On 2nd day after feeding. Brains of sick mice were harvested and suspension of one brain was made in 0.75% BAPS and centrifuged at 10,000 rpm for 1 hr. at 4° C. Supernetant was collected and inoculated in 1 day infant mice and further passaged in mosquitoes. Head squashes were tested by IFA and found positive. Quick complimentary fixation test was performed to confirm the CHP virus using mosquito bodies. The results indicated that *P. papatasi* is susceptible to CHPV and also can transmit virus to susceptible host.

**Flow chart of transmission of CHPV by *P. papatasi* to susceptible host by oral infection**




## b. Transovarial transmission of CHP virus in *P. papatasi*.

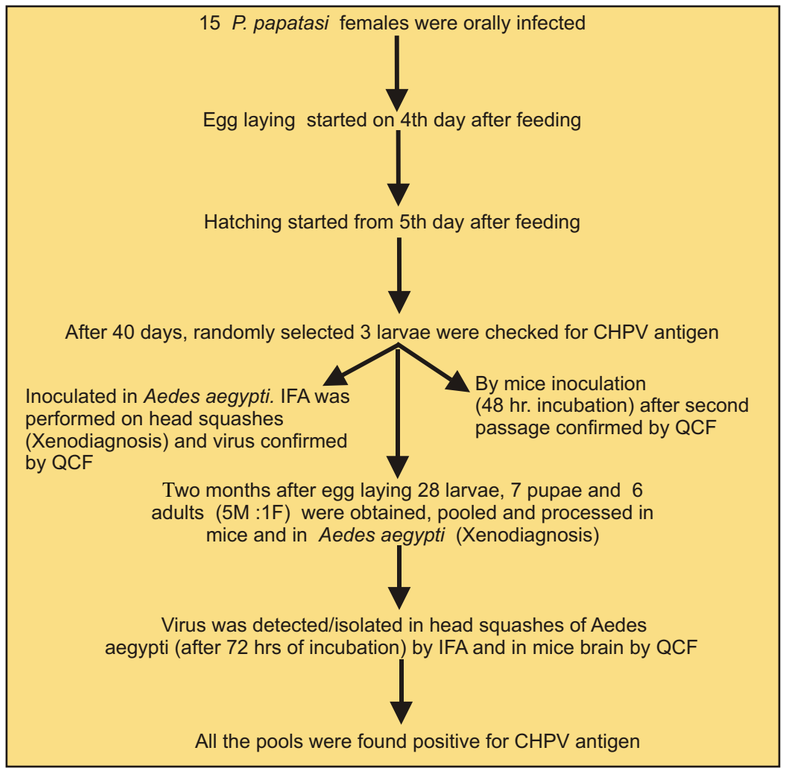
Studies were undertaken to find out the maintenance of the CHP virus in *Phlebetomus papatasi* by transvarial transmission (TOT). For this purpose 50-60 *Phlebetomus papatasi* females were inoculated with CHP virus. These sandflies were fed on normal 1-2 days old infant mice after 24 hours incubation period. On 4th and 5th day after feeding egg laying and hatching was observed respectively. On the 40th day after hatching one pool comprising of 3 larvae were sonicated in 0.75% BAPS and centrifuged at 10,000 rpm for 1 hr. at 4°. Supernetant was collected and inoculated in normal *Ae. aegypti* mosquitoes and infant mice. Mosquito head squashes were tested after 24 hrs. by IFA for presence of CHP virus antigen with positive results. On 2nd PID mice were found sick. Brains were harvested and quick CF test was performed to confirm the CHP virus. On 60th PID 7 Pupae, 28 larvae, 5 adult males and 1 adult female were processed in mosquitoes. Head squashes were tested after 24 hrs by IFA





and found positive except the adult sandfly female. Quick CF using mosquito bodies confirmed CHP virus. The results indicated that *P. papatasi* successfully transmit CHP virus to the progeny transovarially. The virus was observed in larvae, pupae and adults.

**Flow chart of transovarial transmission of CHPV in *P. papatasi* by oral infection**




## 3. Vertical and Venereal transmission of Chandipura virus by Aedes aegypti. Vertical transmission experiment

Inseminated, 35-day-old female mosquitoes were placed in rectangular Barruad cages and starved for 24 h before the infectious meal. The mosquitoes then were allowed to feed on a viremic mouse. Fully engorged females were transferred to holding cages and maintained at 28 ± 2°C and 90 ±10% RH for 2 days for the extrinsic incubation of the virus. An ovicup then was placed in the cage to collect the eggs. After each oviposition, a chick was placed in the cage to allow the mosquitoes to refeed and initiate a new gonotrophic cycle. After 20 days of incubation (3 oviposition cycles), surviving mosquitoes were killed and stored at -70º C until assayed for virus by the Indirect Immuno Florescence Technique (IIFT) to detect CHPV antigen.

Eggs were conditioned for 24 hours to ensure embryonic development and then dried at ambient temperature before being hatched in water. The larvae were reared at 28 ± 2°C. After emergence, the F1 adults were maintained under insectary conditions for 24 h and fed 10% sucrose. After 24 h adults were killed by cold and pooled by sex. Pools of 35  50 individuals were stored at -70º C until assayed for virus. Some of the progeny mosquitoes were examined for CHPV antigen by IIFT using CHPV hyper-immune serum raised in mice. All the pools were passed twice in mice. The presence of virus in the brains of sick mice was confirmed by QCF test.

The maximum likelihood estimation of infection (MLE, number of positive mosquitoes per 100 mosquitoes tested) was calculated with pools of same size using a computer program (Biggerstaff 2003).

Of 515 F1 adult progeny (230 males and 285 females) tested, CHPV was detected in 6 of 11 pools of F1 progeny. Among the pools of infected progeny, 45 % were comprised of males and 55% females (Table 1). The overall vertical infection rate among the F1 progeny was estimated to be 1.2%. The filial infection rates among male and female progeny was 0.9% and 1.4% per 100, respectively, and was not significantly different (p= 0.7). *Ae. aegypti* has never been associated with CHPV in nature, but our laboratory studies showed its vector potential. Although the vertical transmission rates were relatively low compared with results obtained by other investigators, the occurrence of this phenomenon in domestic mosquitoes cannot be understated.

## Venereal transmission experiment

Pupae of *Ae aegypti* were isolated in glass test tubes for emergence. Males were inoculated intrathoracically with approximately 0.2 µl of virus suspension (4.2 log / 0.2 µl $MID_{50}$), following the method described by Rosen and Gubler (1974). All the inoculated male mosquitoes were fed 10% glucose solution for 2 days. On day 2-post infection, the head squashes of some inoculated males were examined for CHPV antigen by IFFT. Remaining males were allowed to mate en masse with virgin females of same age in a ratio of 1: 2 (25males: 50 females) in two different cages. On the 6$^{th}$ day after cohabitation, surviving females (about 90%) were grouped [30 to 32 females each] into three cages and maintained on 10% glucose in an insectary with controlled temperature and humidity. On day 8, females from each cage were examined for evidence of infection by IIFT on head squashes; bodies were passed in 1 to 2 d old suckling mice. Here, bodies were triturated in bovine albumin phosphate saline (BAPS) and centrifuged at 10,000 x g for 1 hr at 4°C. The supernatant was inoculated into suckling mice. The presence of virus in the brains of sick mice was confirmed by QCF test in the second mouse passage.





**Maximum likelihood estimation (MLE) of infection of progeny mosquito by vertical transmission. 95% confidence intervals shown within parentheses.**

| No. of Pools | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | No. of individual | Result | MLE (%) | No. of individual | Result | MLE (%) |
| 1. | 35 | -ve | 1.4 (0.69 to 5.95) | 50 | -ve | 0.9 (0.18 to 3.27) |
| 2. | 50 | -ve | | 50 | -ve | |
| 3. | 50 | ‚ve | | 50 | -ve | |
| 4. | 50 | ‚ve | | 50 | ‚ve | |
| 5. | 50 | ‚ve | | 30 | ‚ve | |
| 6. | 50 | ‚ve | | | | |

In the venereal transmission experiment, 100% (10/10) of inoculated males were positive for CHPV. Overall, 33% females were positive for CHPV; range among the 3 cages was 27 to 43% (Table 2). Although the *Ae. aegypti* is not the natural vector for CHPV and the males used in this experiment were infected by inoculation, the demonstration of efficient venereal transmission of CHPV may have epidemiological importance. Further studies are needed to document possible vertical and venereal transmission of CHPV in the natural sandfly hosts.

**Table 2. CHPV virus infection of *Aedes aegypti* females following the exposure to infected 8 day old males**

| Cages | No. positive/no. tested | % |
|---|---|---|
| 1 | 9/32 | 28.1 |
| 2 | 8/30 | 26.67 |
| 3 | 13/30 | 43.3 |
| **Total** | **30/92** | |
| **Overall %** | **32.69** | |

*Ae. aegypti* was never associated with CHPV in nature, but our laboratory studies showed its vector potential. Orally infected *Ae. aegypti* transmitted CHPV vertically to their F1 progeny. Elevated oral infection rates indicated that *Ae. aegypti* were highly susceptible to CHPV (Ilkal et al 1991). Although the vertical transmission rates were relatively low compared with results obtained by other investigators, the occurrence of this phenomenon in domestic mosquito cannot be undermined.

Although the *Ae. aegypti* is not the natural vector for CHPV and the males used in this experiment were infected by inoculation, the demonstration of efficient venereal transmission of CHPV may have epidemiological importance. Further studies are needed to document possible vertical and venereal transmission of CHPV in the natural sandfly hosts.

## Future plan

Study is being continued

## 4. Molecular analysis of encephalitis epidemic associated with chandipura (CHP) virus.

VA Arankalle vaarankalle@yahoo.com
SP Shrotri, AM Walimbe, BL Rao, AC Mishra

### Objective

To assess if distinct mutations in the G (glycoprotein), N (nucleocapsid) and P (phosphoprotein, earlier referred to as NS) genes could be associated with severity of CHPV infection.

### Achievements

During the outbreak investigations, 5 CHP virus isolates were obtained in cell culture. The following table provides details of these isolates. CHP sequences representing 3 encephalitis cases including one fatal case (patient no 2) and two fever cases were compared. G, N and P genes were amplified for all the isolates and sequenced.

**Details of the CHP viral isolates examined**

| Patient No | Place (State) | Isolate (Date of origin) | Cell line | Inoculum category | Clinical |
|---|---|---|---|---|---|
| 1 | KarimNagar (AP) | CIN0327M (July 2003) | MDCK | Throat swab | Encephalitis |
| 1 | Karimnagar (AP) | CIN0327R (July 2003) | RD | Throat swab | Encephalitis |
| 2 | Karimnagar (AP) | CIN0360R (July 2003) | RD | Brain | Encephalitis |
| 2 | Karimnagar (AP) | CIN0360V (July 2003) | Vero | Brain | Encephalitis |
| 3 | Karimnagar (AP) | CIN0331M (July 2003) | MDCK | Throat swab | Encephalitis |
| 4 | Karimnagar (AP) | CIN0309R (July 2003) | RD | Throatswab | Fever |
| 5 | Karimnagar (AP) | CIN0318R (July 2003) | RD | Throat swab | Fever |
| 6 | CHP | CIN6514V* | BS-C-1 | Serum | Fever |
| | (Maharashtra) | (June1965) | | | |

AP = Andhra Pradesh
* 1965 isolate,

### Sequence analysis

#### G gene analysis

Comparison of G gene sequences obtained during this study with the only sequence (CHPV isolated in India in 1965) available in the genebank data base showed (1) an addition of 17 nucleotides after 1457 base (2) additions at 804, 902 and 1558 positions and (3) deletions at 854 and 869 positions. We therefore sequenced the 1965 isolate available with the repository of the Institute. When compared with this sequence, the 2003 epidemic isolates did not exhibit additions / deletions mentioned above. We therefore deposited 1965-CHP sequence in the gene bank. The sequence obtained by us was used for comparison. The epidemic isolates exhibited 97 + 0.3% identity with each other. As compared to the 1965 isolate, the percent nucleotide identity (PNI) varied from 95.6- 96.1%. For CIN0360 and CIN0327 isolates grown in two different cell lines, the PNI was 100 and 99.9% respectively. The following sequence compares partial G gene nucleotide sequence of the virus from clinical samples (n = 3) with the cell-line isolates. Though sequences derived from different clinical samples exhibited unique





mutations, except one substitution in CIN0331M isolate (A1167C), no changes were noted in the isolates when compared with the corresponding sequences from the clinical samples.

```
CIN036514V  CTCAAGTCTT GACCTCAGAG ATTCAGAGGA TTTTGGATTA TTCCTTGTGT  [ 50]
CIN0360-CSF .C..... ..... ..... ....A..... .....  [ 50]
CIN0360V    .C..... ..... ..... ....A..... .....  [ 50]
CIN0360R    .C..... ..... ..... ....A..... .....  [ 50]
CIN0327-TS  ..... .....G... ..... ..... ...T.....  [ 50]
CIN0327R    ..... .....G... ..... ..... ...T.....  [ 50]
CIN0327M    ..... .....G... ..... ..... ...T.....  [ 50]
CIN0331-TS  ..... .....G..A ..... ..... .....  [ 50]
CIN0331M    ..... .....G..A ..... ..... .....  [ 50]

CIN036514V  CAGAACACGT GGGACAAGGT AGAACGCAAA GAGCCGTTGT CTCCATTGGA  [100]
CIN0360-CSF ..... ..... ..... ..... .....  [100]
CIN0360V    ..... ..... ..... ..... .....  [100]
CIN0360R    ..... ..... ..... ..... .....  [100]
CIN0327-TS  ..... .....A.. ..... ..... .....  [100]
CIN0327R    ..... .....A.. ..... ..... .....  [100]
CIN0327M    ..... .....A.. ..... ..... .....  [100]
CIN0331-TS  ..... ..... ..... ..... .....  [100]
CIN0331M    ..... ..... ..... ..... .....  [100]

CIN036514V  TCTAAGCTAT TTGGCATCTA AATCTCCGGG GAAAGGTCTG GCATATACAG  [150]
CIN0360-CSF ...C..... ..... ..... .....C... ........C.  [150]
CIN0360V    ...C..... ..... ..... .....C... ........C.  [150]
CIN0360R    ...C..... ..... ..... .....C... ........C.  [150]
CIN0327-TS  ...C..... ..... ....C..... ..... .....  [150]
CIN0327R    ...C..... ..... ....C..... ..... .....  [150]
CIN0327M    ...C..... ..... ....C..... ..... .....  [150]
CIN0331-TS  ...C..... ..... ..... .....T.. .....  [150]
CIN0331M    ...C..... ..... ..... .....T.. .....  [150]

CIN036514V  TGATAAATGG GACATTGTCA TTTGCCCATA CTAGATACGT GAGGATGTGG  [200]
CIN0360-CSF ..... ..... ..... .C..... .....  [200]
CIN0360V    ..... ..... ..... .C..... .....  [200]
CIN0360R    ..... ..... ..... .C..... .....  [200]
CIN0327-TS  ..... ..... ..... .C..... .....  [200]
CIN0327R    ..... ..... ..... .C..... .....  [200]
CIN0327M    ..... ..... ..... .C..... .....  [200]
CIN0331-TS  ..... ..... ..... .C..... .....  [200]
CIN0331M    ..... ..... ..... .C..... .....  [200]

CIN036514V  ATTGATGGCC CGGTGTTGAA AGAACCTAAA GGCAAAAGGG AATCCCCTAG  [250]
CIN0360-CSF ..... .....A.. .....G... ..... ....T..C..  [250]
CIN0360V    ..... .....A.. .....G... ..... ....T..C..  [250]
CIN0360R    ..... .....A.. .....G... ..... ....T..C..  [250]
CIN0327-TS  ..... ..... ....ATG... ..... ....T.....  [250]
CIN0327R    ..... ..... ....ATG... ..... ....T.....  [250]
CIN0327M    ..... ..... ....ATG... ..... ....T.....  [250]
CIN0331-TS  ..... ..... ....ATG... ..... ....T.....  [250]
CIN0331M    ..... ..... ....ATG... ..... ....T.....  [250]

CIN036514V  TGGGATCTCG AGTGATATTT GGACCCAATG GTTCAAATAT GGGGATATGG  [300]
CIN0360-CSF ..... .....C.... ..... ..... .....  [300]
CIN0360V    ..... .....C.... ..... ..... .....  [300]
CIN0360R    ..... .....C.... ..... ..... .....  [300]
CIN0327-TS  ..... ..... ..... ..... ..... .....  [300]
CIN0327R    ..... ..... ..... ..... ..... .....  [300]
CIN0327M    ..... ..... ..... ..... ..... .....  [300]
CIN0331-TS  ..... ..... ..... ..... ..A..... .....  [300]
CIN0331M    ..... ..... ..... ..... ..A..... .....  [300]

CIN036514V  AGATAGGTCC AAACGGCCTA TTAAAGACAG CAGGAGGGTA CAAATTCCCC  [350]
CIN0360-CSF ..... .....C ..... ..... ..... .....  [350]
CIN0360V    ..... .....C ..... ..... ..... .....  [350]
CIN0360R    ..... .....C ..... ..... ..... .....  [350]
CIN0327-TS  .....C.. ..... ..... ..... ..... .....  [350]
CIN0327R    .....C.. ..... ..... ..... ..... .....  [350]
CIN0327M    .....C.. .....C ..... ..... ..... .....  [350]
CIN0331-TS  ..... ..... ..... ..... ..... .....  [350]
CIN0331M    ..... .....C ..... ..... ..... .....  [350]

CIN036514V  TGGCATCTGA TCGGTATGGG AATTGTTGAC AATGAACTAC ACGAG [395]
CIN0360-CSF ..... ..... ..... .....T... ..... [395]
CIN0360V    ..... ..... ..... .....T... ..... [395]
CIN0360R    ..... ..... ..... .....T... ..... [395]
CIN0327-TS  ..... ..... ..... ..... ..... [395]
CIN0327R    ..... ..... ..... ..... ..... [395]
CIN0327M    ..... ..... ..... ..... ..... [395]
CIN0331-TS  ..... ..... ..... ..... ..... [395]
CIN0331M    ..... ..... ..... ..... ..... [395]
```

Partial G gene nucleotide sequence alignment of three CHP viruses isolated during the outbreak along with the corresponding sequence derived from the clinical samples. For the details of the isolates please refer the table. The accession numbers for the sequences derived from clinical specimens and published earlier (1) are AY554407, AY554409, AY554411)

Alignment of deduced amino acid sequences of the G protein (530 amino acids) from different isolates is shown in figure below. A total of 7 Amino acid substitutions were noted for the epidemic isolates (amino acid change from Leucine (L) ➔ Serine (S) at position 19, Tyrosine (Y) ➔ Serine (S) at position 22, Threonine (T) Alanine (A) at position 219, Glycine (G) ➔ Alanine (A) at position 222, Arginine (R) ➔ Lysine (K) at position 264, Histidine (H) ➔ Proline (P) at position 269 and Threonine (T) ➔ Alanine (A) at position 279). In addition, the brain-derived isolate exhibited additional four substitutions; Isoleucine (I) ➔ Valine (V) at position 16, Asparagine (N) ➔ Serine (S) at position 30, Isoleucine (I)➔ Valine (V) at position 218, Arginine (R) ➔ Lysine (K) at position 502. This isolate did not replace Proline (P)➔ Methionine (M) at position 367 seen in other epidemic isolates.

Two amino acid substitutions were seen in the isolates from encephalitis cases (CIN0327M & CIN0327R) at position 40 (Lysine (K) ➔Arginine (R)) and position 424 (Leucine (L)➔Valine (V)). One fever case-derived isolate CIN0309R showed an additional substitution at position 213 A, Aspartic Acid (D)➔Valine (V).

```
CIN036514V ---------V LLISFITPLY SYLSIAFPEN TKLDWKPVTK NTRYCPMGGE    [ 50]
CIN0360V   ---------- .....V..S. .S.......S .......... ..........    [ 50]
CIN0327M   ---------. ........S. .S........ .........R ..........    [ 50]
CIN0327R   ---------. ........S. .S........ .........R ..........    [ 50]
CIN0331M   ---------- ........S. .S........ .......... ..........    [ 50]
CIN0318R   ---------- ........S. .S........ .......... ..........    [ 50]
CIN0309R   MTSSVTISV. ........S. .S........ .......... ..........    [ 50]

           Signal sequence

CIN036514V WFLEPGLQEE SFLSSTPIGA TPSKSDGFLC HAAKWVTTCD FRWYGPKYIT    [100]
CIN0360V   .......... .......... .......... .......... ..........    [100]
CIN0360R   .......... .......... .......... .......... ..........    [100]
CIN0327M   .......... .......... .......... .......... ..........    [100]
CIN0327R   .......... .......... .......... .......... ..........    [100]
CIN0331M   .......... .......... .......... .......... ..........    [100]
CIN0318R   .......... .......... .......... .......... ..........    [100]
CIN0309R   .......... .......... .......... .......... ..........    [100]

CIN036514V HSIHNIKPTR SDCDTALASY KSGTLVSPGF PPESCGYASV TDSEFLVIMI    [150]
CIN0360V   .......... .......... .......... .......... ..........    [150]
CIN0360R   .......... .......... .......... .......... ..........    [150]
CIN0327M   .......... .......... .......... .......... ..........    [150]
CIN0327R   .......... .......... .......... .......... ..........    [150]
CIN0331M   .......... .......... .......... .......... ..........    [150]
CIN0318R   .......... .......... .......... .......... ..........    [150]
CIN0309R   .......... .......... .......... .......... ..........    [150]

CIN036514V TPHHVGVDDY RGHWVDPLFV GGECDQSYCD TIHNSSVWIP ADQTKKNICG    [200]
CIN0360V   .......... .......... .......... .......... ..........    [200]
CIN0360R   .......... .......... .......... .......... ..........    [200]
CIN0327M   .......... .......... .......... .......... ..........    [200]
CIN0327R   .......... .......... .......... .......... ..........    [200]
CIN0331M   .......... .......... .......... .......... ..........    [200]
CIN0318R   .......... .......... .......... .......... ..........    [200]
CIN0309R   .......... .......... .......... .......... ..........    [200]

CIN036514V QSFTPLTVTV AYDKTKEITA GGIVFKSKYH SHMEGARTCR LSYCGRNGIK    [250]
CIN0360V   .......... .......VA. .A........ .......... ..........    [250]
CIN0360R   .......... .......VA. .A........ .......... ..........    [250]
CIN0327M   .......... ........A. .A........ .......... ..........    [250]
CIN0327R   .......... ........A. .A........ .......... ..........    [250]
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```
CIN0331M   .......... ........A. .A........ .......... ..........    [250]
CIN0318R   .......... ........A. .A........ .......... ..........    [250]
CIN0309R   .......... ..V.....A. .A........ .......... ..........    [250]

CIN036514V FPNGEWVSLD VKTRIQEKHL LPLFKECPTG TEVRSTLQSD GAQVLTSEIQ    [300]
CIN0360V   .......... ...K....P. ........A. .......... ..........    [300]
CIN0360R   .......... ...K....P. ........A. .......... ..........    [300]
CIN0327M   .......... ...K....P. ........A. .......... ..........    [300]
CIN0327R   .......... ...K....P. ........A. .......... ..........    [300]
CIN0331M   .......... ...K....P. ........A. .......... ..........    [300]
CIN0318R   .......... ...K....P. ........A. .......... ..........    [300]
CIN0309R   .......... ...K....P. ........A. .......... ..........    [300]

CIN036514V RILDYSLCQN TWDKVERKEP LSPLDLSYLA SKSPGKGLAY TVINGTLSFA    [350]
CIN0360V   .......... .......... .......... .......... ..........    [350]
CIN0360R   .......... .......... .......... .......... ..........    [350]
CIN0327M   .......... .......... .......... .......... ..........    [350]
CIN0327R   .......... .......... .......... .......... ..........    [350]
CIN0331M   .......... .......... .......... .......... ..........    [350]
CIN0318R   .......... .......... .......... .......... ..........    [350]
CIN0309R   .......... .......... .......... .......... ..........    [350]

CIN036514V HTRYVRMWID GPVLKEPKGK RESPSGISSD IWTQWFKYGD MEIGPNGLLK    [400]
CIN0360V   .......... .......... .......... .......... ..........    [400]
CIN0360R   .......... .......... .......... .......... ..........    [400]
CIN0327M   .......... ......M... .......... .......... ..........    [400]
CIN0327R   .......... ......M... .......... .......... ..........    [400]
CIN0331M   .......... ......M... .......... .......... ..........    [400]
CIN0318R   .......... ......M... .......... .......... ..........    [400]
CIN0309R   .......... ......M... .......... .......... ..........    [400]

CIN036514V TAGGYKFPWH LIGMGIVDNE LHELSEANPL DHPQLPHAQS IADDSEEIFF    [450]
CIN0360V   .......... .......... .......... .......... ..........    [450]
CIN0360R   .......... .......... .......... .......... ..........    [450]
CIN0327M   .......... .......... ...V...... .......... ..........    [450]
CIN0327R   .......... .......... ...V...... .......... ..........    [450]
CIN0331M   .......... .......... .......... .......... ..........    [450]
CIN0318R   .......... .......... .......... .......... ..........    [450]
CIN0309R   .......... .......... .......... .......... ..........    [450]

CIN036514V GDTGVSKNPV ELVTGWFTSW KESLAAGVVL ILVVVLIYGV LRCFPVLCTTCR  [502]
CIN0360V   .......... .......... .......... .......... ..........K  [502]
CIN0360R   .......... .......... .......... .......... ..........K  [502]
CIN0327M   .......... .......... .......... .......... ..........  [502]
CIN0327R   .......... .......... .......... .......... ..........  [502]
CIN0331M   .......... .......... .......... .......... ..........  [502]
CIN0318R   .......... .......... .......... .......... ..........  [502]
CIN0309R   .......... .......... .......... .......... ..........  [502]

                                  Transmembrane Region

CIN036514V KPKWKKGV ERSDSFEMRI FKPNNMRARV  [530]
CIN0360V   ........ .......... ..........  [530]
CIN0360R   ........ .......... ..........  [530]
CIN0327M   ........ .......... ..........  [530]
CIN0327R   ........ .......... ..........  [530]
CIN0331M   ........ .......... ..........  [530]
CIN0318R   ........ .......... ..........  [530]
CIN0309R   ........ .......... ..........  [530]

           Intracytoplasmic Region
```

Alignment of the deduced amino acid sequences of the G protein of different isolates of CHP virus. For the details of the isolates please refer the table. Solid bars represent signal sequence (1-18 aa), transmembrane region sequence (482-502 aa) and the intracytoplasmic region sequence (503-530).

## N Gene analysis

The 1965 isolate was 96.5-97.6% identical with the epidemic isolates whereas the epidemic isolates were 97.7 + 0.3% identical with each other. The isolates grown in different cell lines exhibited 99.3% (CIN0360) and 99.5% (CIN0327) identity at nucleotide level. A single amino acid substitution, Lysine (K) Arginine (R) at position 37 was noted for all the epidemic isolates. In all isolates except CIN0331M, Aspartic Acid (D) at position 364 substituted Glutamic acid

(E). Additional substitutions (Valine (V) Isoleucine (I) at position 413) in the brain derived isolate and Alanine (A) Threonine (T) at position 163 in CIN0309R were present as shown below.

```
CIN036514V MSSQVFCIST GQTVSVCLPA NEDPVEFPGA FFTPNAKKPT VYIKKETDLS  [ 50]
CIN0360V   .......... .......... .......... ......R... ..........  [ 50]
CIN0360R   .......... .......... .......... ......R... ..........  [ 50]
CIN0327M   .......... .......... .......... ......R... ..........  [ 50]
CIN0327R   .......... .......... .......... ......R... ..........  [ 50]
CIN0331M   .......... .......... .......... ......R... ..........  [ 50]
CIN0318R   .......... .......... .......... ......R... ..........  [ 50]
CIN0309R   .......... .......... .......... ......R... ..........  [ 50]

CIN036514V LLRSHVYDGI KDGSVTVSQI NSYLYMVLKD IREKPDKNWT SFGVELGKKN  [100]
CIN0360V   .......... .......... .......... .......... ..........  [100]
CIN0360R   .......... .......... .......... .......... ..........  [100]
CIN0327M   .......... .......... .......... .......... ..........  [100]
CIN0327R   .......... .......... .......... .......... ..........  [100]
CIN0331M   .......... .......... .......... .......... ..........  [100]
CIN0318R   .......... .......... .......... .......... ..........  [100]
CIN0309R   .......... .......... .......... .......... ..........  [100]

CIN036514V EPMGIFDLLN VEDVKGKELD KKGQDTRLPG DDLWLPTLIF GLYRVSRATQ  [150]
CIN0360V   .......... .......... .......... .......... ..........  [150]
CIN0360R   .......... .......... .......... .......... ..........  [150]
CIN0327M   .......... .......... .......... .......... ..........  [150]
CIN0327R   .......... .......... .......... .......... ..........  [150]
CIN0331M   .......... .......... .......... .......... ..........  [150]
CIN0318R   .......... .......... .......... .......... ..........  [150]
CIN0309R   .......... .......... .......... .......... ..........  [150]

CIN036514V VEYKKTLMTN LYAQCKLRTK DAEEIVDETA EFFNAWANDS NFTKIVAAVD  [200]
CIN0360V   .......... .......... .......... .......... ..........  [200]
CIN0360R   .......... .......... .......... .......... ..........  [200]
CIN0327M   .......... .......... .......... .......... ..........  [200]
CIN0327R   .......... .......... .......... .......... ..........  [200]
CIN0331M   .......... .......... .......... .......... ..........  [200]
CIN0318R   .......... .......... .......... .......... ..........  [200]
CIN0309R   .......... ..T....... .......... .......... ..........  [200]

CIN036514V MYFHHFKKSD HAPIRFGTIV SRFKDCAALS TLSHLQKVTG LPIEEVFTWV  [250]
CIN0360V   .......... .......... .......... .......... ..........  [250]
CIN0360R   .......... .......... .......... .......... ..........  [250]
CIN0327M   .......... .......... .......... .......... ..........  [250]
CIN0327R   .......... .......... .......... .......... ..........  [250]
CIN0331M   .......... .......... .......... .......... ..........  [250]
CIN0318R   .......... .......... .......... .......... ..........  [250]
CIN0309R   .......... .......... .......... .......... ..........  [250]

CIN036514V FNKSVQDDLL RMMTPGQEID QADSYMPYLI DMGLSTKSPY SSTKNPSFHF  [300]
CIN0360V   .......... .......... .......... .......... ..........  [300]
CIN0360R   .......... .......... .......... .......... ..........  [300]
CIN0327M   .......... .......... .......... .......... ..........  [300]
CIN0327R   .......... .......... .......... .......... ..........  [300]
CIN0331M   .......... .......... .......... .......... ..........  [300]
CIN0318R   .......... .......... .......... .......... ..........  [300]
CIN0309R   .......... .......... .......... .......... ..........  [300]

CIN036514V WGQLTAFLVK SARAKNALVP VDIAYHELTT AALLFAYAIG RSSELEQRFV  [350]
CIN0360V   .......... .......... .......... .......... ..........  [350]
CIN0360R   .......... .......... .......... .......... ..........  [350]
CIN0327M   .......... .......... .......... .......... ..........  [350]
CIN0327R   .......... .......... .......... .......... ..........  [350]
CIN0331M   .......... .......... .......... .......... ..........  [350]
CIN0318R   .......... .......... .......... .......... ..........  [350]
CIN0309R   .......... .......... .......... .......... ..........  [350]

CIN036514V LNGKKFTKEK DSREDNDTTP PSERNVVVWL AWWEDIKHEI TPDMKAFAKR  [400]
CIN0360V   .......... ...D...... .......... .......... ..........  [400]
CIN0360R   .......... ...D...... .......... .......... ..........  [400]
CIN0327M   .......... ...D...... .......... .......... ..........  [400]
CIN0327R   .......... ...D...... .......... .......... ..........  [400]
CIN0331M   .......... .......... .......... .......... ..........  [400]
CIN0318R   .......... ...D...... .......... .......... ..........  [400]
CIN0309R   .......... ...D...... .......... .......... ..........  [400]

CIN036514V AVERVGDIRV NSVAEYARKL FA* [423]
CIN0360V   .......... ..I....... ... [423]
CIN0360R   .......... ..I....... ... [423]
```





```
CIN0327M   .......... .......... ... [423]
CIN0327R   .......... .......... ... [423]
CIN0331M   .......... .......... ... [423]
CIN0318R   .......... .......... ... [423]
CIN0309R   .......... .......... ... [423]
```

Alignment of the deduced amino acid sequences of the N protein of different isolates of CHP virus. For the details of the isolates please refer the table.

## P gene analysis

Among epidemic isolates, the PNI was 97.4 + 0.4% whereas 95.8-96.8% identity was observed with the 1965 isolate. CIN0360 and CIN0327 isolates grown in different cell lines were 99.7 and 99% identical at nucleotide level respectively. Glutamic acid (E)➔ Aspartic Acid (D) substitution at position 64 was present in all the epidemic isolates. A unique single amino acid substitution was noted for three isolates; Glutamine(Q)➔ Arginine (R) at position 103 in CIN0309R (fever case), Isoleucine (I)➔ Valine (V) at position 180 in brain derived isolates and Asparagine(N)➔ Threonine(T) at position 257 in CIN0327M (encephalitis case). In addition, Glycine (G) ➔Glutamic Acid (E) substitution at position 112 was recorded in CIN0327R, CIN0327M, CIN0309R, CIN0331M isolates. Alanine (A) ➔Valine (V) at position 214 in all except CIN0327R, CIN0360R and Isoleucine (I)➔Valine (V) at position 270 in CIN0318R, CIN0309R, CIN0331M were also present as shown in the following sequence.

```
CIN036514V MEDSQLYQAL KNYPKLQDTL DSIENLEDDT KSEPSECGSP TERGIPSYYL  [ 50]
CIN0360V   .......... .......... .......... .......... ..........  [ 50]
CIN0360R   .......... .......... .......... .......... ..........  [ 50]
CIN0327M   .......... .......... .......... .......... ..........  [ 50]
CIN0327R   .......... .......... .......... .......... ..........  [ 50]
CIN0331M   .......... .......... .......... .......... ..........  [ 50]
CIN0318R   .......... .......... .......... .......... ..........  [ 50]
CIN0309R   .......... .......... .......... .......... ..........  [ 50]

CIN036514V AEELDECEEE DSEEDDDNLP TEIPDPPTVD MLEAIMEDEI DDTAYQVHFE  [100]
CIN0360V   .......... ...D...... .......... .......... ..........  [100]
CIN0360R   .......... ...D...... .......... .......... ..........  [100]
CIN0327M   .......... ...D...... .......... .......... ..........  [100]
CIN0327R   .......... ...D...... .......... .......... ..........  [100]
CIN0331M   .......... ...D...... .......... .......... ..........  [100]
CIN0318R   .......... ...D...... .......... .......... ..........  [100]
CIN0309R   .......... ...D...... .......... .......... ..........  [100]

CIN036514V AKQTWKPVIE TGGNERGKFT LSVPQNLSAL QLLQWETGIH ALAERLGGCR  [150]
CIN0360V   .......... .......... .......... .......... ..........  [150]
CIN0360R   .......... .......... .......... .......... ..........  [150]
CIN0327M   .......... .E........ .......... .......... ..........  [150]
CIN0327R   .......... .E........ .......... .......... ..........  [150]
CIN0331M   .......... .E........ .......... .......... ..........  [150]
CIN0318R   .......... .......... .......... .......... ..........  [150]
CIN0309R   ..R....... .E........ .......... .......... ..........  [150]

CIN036514V LLQISTRGTR DGIEFTVRET PCVSPASDPI PSTSRSSSIA SNVSTRQTES  [200]
CIN0360V   .......... .......... .........V .......... ..........  [200]
CIN0360R   .......... .......... .........V .......... ..........  [200]
CIN0327M   .......... .......... .......... .......... ..........  [200]
CIN0327R   .......... .......... .......... .......... ..........  [200]
CIN0331M   .......... .......... .......... .......... ..........  [200]
CIN0318R   .......... .......... .......... .......... ..........  [200]
CIN0309R   .......... .......... .......... .......... ..........  [200]

CIN036514V PGSKSNTSLG IPEAPANLID MGAIDKEFIL AAISPSDPPY KNTLRNLFGS  [250]
CIN0360V   .......... ...V...... .......... .......... ..........  [250]
CIN0360R   .......... .......... .......... .......... ..........  [250]
CIN0327M   .......... ...V...... .......... .......... ..........  [250]
CIN0327R   .......... .......... .......... .......... ..........  [250]
CIN0331M   .......... ...V...... .......... .......... ..........  [250]
CIN0318R   .......... ...V...... .......... .......... ..........  [250]
CIN0309R   .......... ...V...... .......... .......... ..........  [250]

CIN036514V GDSFEQYNQT GIYSLKELVI AGLKRKGIYN RIRIRCHLEP QFN* [294]
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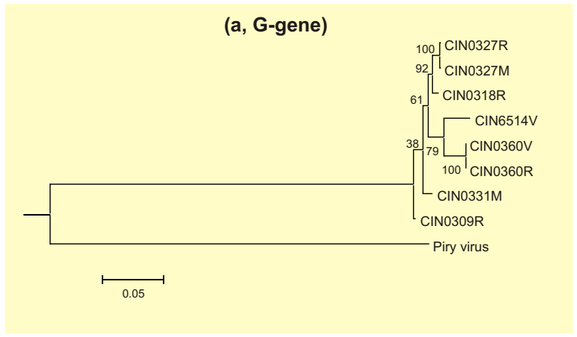
CIN0360V   .......... .......... .......... .......... .... [294]
CIN0360R   .......... .......... .......... .......... .... [294]
CIN0327M   .......T.. .......... .......... .......... .... [294]
CIN0327R   .......... .......... .......... .......... .... [294]
CIN0331M   .......... .........V .......... .......... .... [294]
CIN0318R   .......... .........V .......... .......... .... [294]
CIN0309R   .......... .........V .......... .......... .... [294]
```

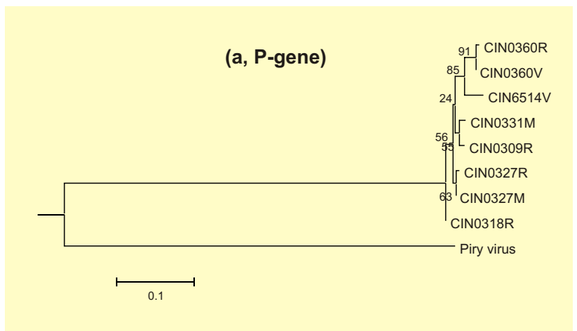
Alignment of the deduced amino acid sequences of the P protein of different isolates of CHP virus. For the details of the isolates please refer the table.

## Phylogenetic analysis

Phylogenetic status of different epidemic isolates on the basis of G, N and P gene sequences is given in the following trees. In All analyses, the brain-derived isolate clustered with the 1965 isolate with 100% bootstrap support. The other cluster was further subdivided into two with sequences from one encephalitis and one fever case grouping together in both the subgroups. As seen from figures b and c, almost identical groupings were obtained on the basis of N and P gene-based phylogenetic analyses.

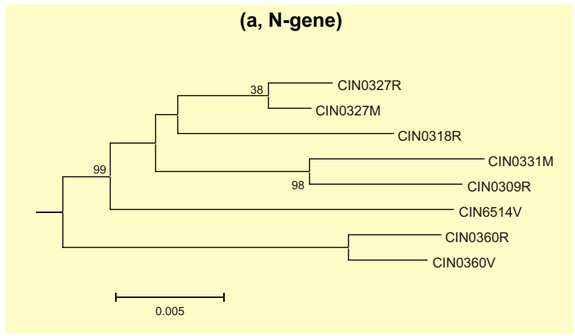
In conclusion, the present study shows that CHP viruses isolated from human cases in India in 1965 and 2003 were not much divergent. Though several amino acid substitutions were recorded in G protein, the significance of these mutations in the pathogenesis of CHP infection and probable encephalitis epidemic potential of the virus with very high mortality needs to be immediately examined.








Phylogenetic analyses of complete G gene (a), N gene (b) and P gene (c) of CHP isolates. Refer to table for the details of the isolates used. Percent bootstrap support is indicated by the values at each node. For G and P-gene-based analyses, Piry virus was used as an outgroup. For N-gene, unrooted tree was constructed, as sequence for Piry virus was not available.

## 5. Th1 cytokine profile and possible involvement of IL 2 in recovery from chandipura virus infection

**AS Tripathy** anuradhastripathy@hotmail.com
**Balaji S, Narsimha A, Mishra AC, Arankalle VA**

### Objectives

To assess the role of cytokines in the pathogenesis of CHP virus infection

### Achievements

Investigations were undertaken during the declining phase of the CHP virus epidemic. Fourteen children presenting with encephalitis and admitted to a tertiary care hospital were studied. Virus specific IgM antibodies could be detected in 1/5 patient bled within 4 days of onset of clinical symptoms and 8/9 patients bled after 4 days. Concentrations of IL-2, IFN-, IL-6 and TNF- in mitogen stimulated peripheral blood mononuclear cell culture (PBMC) supernatants were assessed in ELISA. IFN- levels were significantly elevated as against IL-2 levels (441.69+126.05 vs 47.32+41.36, P<0.01) in children within 4 days of post onset of disease. There was no difference in IFN- and IL-2 levels in children who were bled after 4 days post onset. Significant levels of IL-2 in the later state of disease compared to that in early state of disease (282.73+307.64 vs 47.32+41.36, P<0.05) suggests the possible role of IL-2 in recovery. High levels of IFN- along with significantly high levels of IL-2 in the later state of the disease suggest the role of Th1 cells in recovery. Absence of variations in IL-6 levels in both the groups indicate the absence of its specific role in disease progression or recovery. Non-significantly elevated levels TNF- in the later stage of the disease compared to the early stage, where mortality is high, suggests its role in reducing virus titres.





## 6. Increased frequency of HLAA28 allele among CHP encephalitis patients

**AS Tripathy** anuradhastripathy@hotmail.com
**Balaji S, Mishra AC, Arankalle VA**

### Objectives

To assess the association of class I HLA alleles with CHP encephalitis

### Achievements

HLA A, B and Cw allele distribution was determined among 14 children with clinically and serologically confirmed CHP encephalitis and the results were compared with 385 ethnically matched apparently healthy individuals. Genomic DNA was extracted from frozen peripheral blood mononuclear cells. For HLA A, B and C typing PCR-SSP molecular method was followed. To overcome chance deviate frequency of HLA allele, the p value was corrected by the use of Bonferroni inequality method. The HLA A28, A3 alleles were more commonly observed among CHP infected individuals when compared with controls. In fact, A28 was significantly increased among CHP virus infected children when compared with controls ($p<0.0001$) suggesting involvement of HLA class I alleles in the genetic susceptibility to CHP associated encephalitis. Further molecular subtyping of A28 gene would help to understand the influence of haplotype association and other intervening genes within the MHC complex.

## 7. Development of inactivated tissue culture derived vaccine for CHP virus

**Jadi RS**
**Mishra AC**

Recently, episodes of encephalitis were reported from the states of Andhra Pradesh and Maharashtra affecting children of age group ranging from 4 to 12 years with the mortality rate of approximately 55%. It is proposed to initiate studies for the development of tissue culture based inactivated CHP vaccine for the prevention of this disease.

### Objectives

- To establish animal model for CHP virus.
- Development of inactivated tissue culture derived vaccine

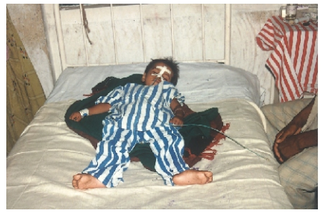
### Achievement

**Studies on animal model for CHP virus**

Experiments were designed and conducted to study animal model for CHPV. Swiss albino mice of different age group (2 day to 3 weeks old) were inoculated with known amount of CHP virus by ip & ic route and observed for sickness. It was observed that mice of all age groups inoculated by ic died within 24 to 72 hours of incubation period. Mice inoculated by ip showed differential sickness. All one-week old mice died 72 hours post infection, whereas, two-week old mice died 188 hours post infection. Only one of the five three-week old mice died 164 hours post infection. Further experiments are being undertaken to determine cut of age for susceptibility.

# Japanese Encephalitis/ West Nile

Mosquito collection by chick baited trap



A JE patient in hospital

Vectors of JE : Cattle tethered mosquito collection at dusk

## 1. Entomological and epidemiological investigation of an outbreak of encephalitis of suspected viral aetiology among humans in Bhandara and Nagpur districts.

**PC Kanojia** kanojiapc@yahoo.co.in

Investigation of epidemics of encephalitis of suspected viral etiology are reported from various areas. Investigations in endemic areas were carried out to during June July 2003

### Objectives

- To determine the aetiological agent of the epidemic.
- To study the vectors of JE, WN and CHPV.

### Achievements

#### Bhandara

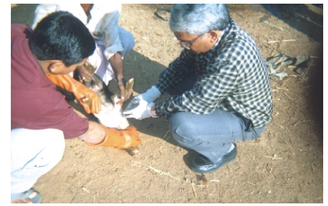
During June-July, 2003, 30 encephalitis cases with 16 deaths were reported from Bhandara district Maharashtra. Eleven deaths out of 16 cases occurred within 24 hours of the seizure. Entomological investigation was carried out in 8 affected localities. Out of 2192 mosquitoes belonging to 21 species collected, *Cx.tritaeniorhynchus* was the most predominant species and accounted for 31.4% followed by *An.subpictus* 22.7% and *Cx.fuscocephala* 19.3%. Sixty-four mosquito pools were prepared from field caught specimens to isolate the aetiological agent. Sandflies were also collected from affected villages, resting inside the houses. A total of 94 sandflies belonging to 3 species in 2 genera (*Phlebotomus and Sergentomyia)* were collected during the investigation. Species belonging to the genus *Sergentomyia* were collected in large numbers. Whereas species belonging to genus *Phlebotomus* were collected in very small numbers. In all, 22 sandflies pools were prepared from field caught specimens and were tested in cell culture for the presence of CHP virus. All the pools were found negative.

#### Nagpur

During June-July 2003, encephalitis cases were reported from Nagpur district. Forty cases in pediatric age group with 27 deaths were registered between 26$^{th}$ June and 29$^{th}$ July, 2003. Most of the deaths occurred within 24 hours of the seizure. Total 789 mosquitoes belonging to 15 species were collected at dusk. *An.subpictus* was the most abundant species and accounted for 45% followed by *Cx.tritataeniorhynchus* (13.2%) and *An.culicifascies* (10%). Besides mosquitoes, 16 sandflies were collected from affected villages amongst them *Sergentomyia* species were the most predominant species.







JE virus amplifier :
Pig-blood sample collection

## 2. Surveillance of JE/WN vectors and vertebrate hosts in Bellary district, Karnataka state.

**PC Kanojia** kanojiapc@yahoo.com

Surveillance of vectors and vertebrate hosts in an endemic area is necessary to understand changing patterns in the host vector relationship.

### Objectives

- To incriminate vector species by virus isolation attempts and population dynamics studies.
- To determine the role of vertebrate host in the natural cycle of JE in the district.

### Achievements

#### Serological survey of vertebrate hosts

##### Birds

In order to determine JE/WN virus activity in the avian fauna prevailing in the Bellary district, serological survey of birds was carried out in November 2003. A total of 159 sera were collected from different species of birds. HI test was conducted on 158 sera. Six Paddy birds and three cattle egrets were found positive for WN virus. Two paddy birds were found positive for JE as well as WN viruses. 147 sera were negative.

##### Pigs

In order to monitor JE/WN virus activity in amplifying hosts, 59 pig sera were collected from JE affected villages during the epidemic season. These sera were tested for HI antibodies. Results of HI tests evidenced positivity for JE in 5 sera, JE+ WN in 35 and none in 18. One sample was not tested.

#### Entomological studies

##### Day time collection

A total of 12054 mosquitoes belonging to 4 genera and 13 species were collected from indoor resting sites such as human dwelling and animal sheds from January to August, 2003. Out of 13 species, 3 species viz., *Culex tritateniorhynchus* (47.8%), *Anopheles subpictus* (35.3%) and *Cx.quinquefasciatus* (14.3%) formed 97.4% of the total catch. Remaining 10 species contributed only 2.6% in the entire collection. Per man-hour density (PMHD) of *Cx.tritaeniorhynchus* was highest (96) in February and lowest (39.7) in March.

##### Dusk collection

A total 20448 female mosquitoes belonging to 16 species were collected from in and around animal sheds. *Cx.tritaeniorhynchus* was the most abundant species and accounted for 72.1% of the total catch followed by *An.subpictus* (19.8%), *Cx. quinquefasciatus* (5%) and *An.peditaeniatus* (2.2%). Remaining 12 species contributed < 1% in the total catch. Peak density of mosquitoes, particularly *Cx.tritateniorhynchus* coincided with the incidence of JE in the district as in the previous year.

##### Larval collection

In all 4231 mosquito larvae representing 3 genera and 11 species were sampled from different

Birds are important hosts for JE and WN Viruses

breeding habitats. *Cx.tritateniorhynchus* contributed significantly in the total collection and its percentage was recorded 52.45 followed by *An.subpictus/vagus* 43.7 and *Cx.bitaeniorhynchus* 2.4%. Maximum number of *Cx.tritateniorhynchus* larvae were sampled during January and February from paddy fields.

## Incidence of JE in humans

Six cases of JE were reported from different localities of Bellary district in January 2003. No cases were reported from February to August, 2003.

## Human serology

One hundred fifty human sera, particularly from school going children were collected from different localities in order to detect prevalence of JE / WN antibodies. Results HI tests on these sera showed JE positivity in 10, WN in 18, JE& WN in 47 and none in 75.

## Virus isolation attempts

A total 31 mosquito pools collected from JE affected villages of Belllary district were processed for the detection of virus by antigen capture ELISA. Six pools of *Cx. tritaeniorhynchus* and one pool of *Cx.quinquefasciatus* were found positive. These positive samples were passaged into two day old mice' which yielded negative results when tested by ELISA and RT-PCR.





## 3. Etiological, epidemiological and entomological studies on flavivirus infections in Karnataka and neighbouring areas with particular reference to JE, WN and DEN viruses

**P N Yergolkar** nivbng@bgl.vsnl.net.in
**P George Jacob**

JE, WN and DEN viral infections in Karnataka and neighbouring areas have remained important public health problems. There is need need to continuously monitor for early diagnosis, patient management and undertaking prevention and control measures.

## Objectives

- To diagnose and establish JE, WN and DEN viral infections in suspected patients in sporadic, endemic and epidemic form.
- To study epidemiological features, establish serum/virus bank and data base for prevention and control of diseases particularly JE and newly emerging Dengue Haemorrhagic Fever (DHF), Dengue Shock Syndrome (DSS), rural and urban dengue fever outbreaks.
- To provide laboratory diagnosis to the concerned physicians for patient management and to the State and District Health authorities for undertaking necessary prevention and control measures.
- Bio-ecological studies on mosquito vectors of JE/WN and DEN viruses.

Paddy fields with stagnant water support proliferation of JE vector mosquitoes

Swamps and puddles support breeding of some mosquitoe vector species

## Achievements

### Studies on JE in Karnataka and neighbouring districts of Andhra Pradesh, Tamil Nadu & Kerala.

During the report period from April 2003 to March 2004 a total of 264 specimens (131 CSF and 133 sera) from 140 suspected cases of encephalitis were received. Of these cases, 12 cases had paired CSF and or paired serum, 96 single CSF and serum, 18 single CSF and 14 single serum samples received for diagnosis. All specimens were tested for IgM antibodies to JE by MAC-ELISA. Out of 140 cases JE diagnosis was made in 44 cases and 96 were negative. Area wise positivity is shown in the table.

Serological diagnosis of suspected encephalitis cases from Karnataka and neighbouring districts of Andhra Pradesh, Tamil Nadu & Kerala, Apr-2003 to Mar-2004.

| Sl. No. | District/State | No. of cases tested | No. of cases Pos. for JE | Negative |
|---|---|---|---|---|
| 1 | Bellary - Karnataka | 31 | 15 | 16 |
| 2 | Kolar | 34 | 10 | 24 |
| 3 | Koppal | 8 | 6 | 2 |
| 4 | Raichur | 13 | 5 | 8 |
| 5 | Bangalore (Urban) | 7 | 1 | 6 |
| 6 | Bangalore (Rural) | 4 | 1 | 3 |
| 7 | Belgaum | 1 | 1 | - |
| 8 | Davanagere | 8 | 1 | 7 |
| 9 | Bangalore City | 5 | - | 5 |
| 10 | Chitradurga | 1 | - | 1 |
| 11 | Dharwad | 4 | - | 4 |
| 12 | Hassan | 1 | - | 1 |
| 13 | Haveri | 1 | - | 1 |
| 14 | Tumkur | 1 | - | 1 |
| **Total - Karnataka** | | **119** | **40** | **79** |
| 15 | Anantapur - Andhra Pradesh | 6 | 3 | 3 |
| 16 | Chittoor | 2 | - | 2 |
| 17 | Nellore | 8 | - | 8 |
| 18 | Guntur | 1 | - | 1 |
| **Total - Andhra Pradesh** | | **17** | **3** | **14** |
| 19 | Salem - Tamil Nadu | 1 | 1 | - |
| 20 | Kerala | 1 | - | 1 |
| 21 | Address not known | 2 | - | 2 |
| **Grand total** | | **140** | **44** | **96** |

JE positive cases were diagnosed in the months of June-1, October-5, November-28, December-6 and 4 in January 2004.

Seasonal distribution of encephalitis and confirmed JE cases

JE cases occurred predominantly (93.2%) in the pediatric group.





Age & Sex wise distribution of Suspected Encephalitis Cases, from Karnataka and bordering districts of Andhra Pradesh, Tamil Nadu & Kerala, Apr-2003 to Mar-2004.

| Age group (yrs) | Male | Female | Total |
|---|---|---|---|
| <1 5 | *14/53 | 5/20 | 19/73 |
| 6 10 | 10/20 | 12/26 | 22/46 |
| 11- 15 | 2/9 | 0/5 | 2/14 |
| 16 65 | 1/3 | 0/3 | 1/6 |
| Age not known | 0/1 | - | 0/1 |
| Total | 27/86 | 17/54 | 44/14 |

** No. Pos. for JE/Total Tested.*

## Future plan

There is a need for continuous surveillance and diagnosis of JE cases for undertaking preventive control measures and better management of cases

## 4. Tissue culture derived inactivated vaccine against JE virus.

MM Gore milind_gore@hotmail.com
VM Ayachit

In order to have a effective Indian vaccine, it is necessary to have a strain that can induce neutralizing antibodies against majority of JE virus strains available in India. Work was initiated to select a set of strains suitable for tissue culture derived vaccine.

## Objective

To select JE virus strain capable of inducing protective activity against different JE virus isolates from India.

## Achievements

Based on the earlier results, feasibility studies of carrying out chick embryo culture derived inactivated JE virus vaccine have been initiated.

JE virus strains isolated from Lakhimpur were passaged in Chick embryo cultures provided by Serum Institute of India. JE virus strain 014173 did not result in good titres. Two passages of JE virus strain 014178 has resulted in obtaining virus titre of 10^6.

## Future plan

Work on further adaptation and other GMP requirements would be taken up.

## 5. Development of chimeric Th-B cell peptide vaccine against JE virus

**MM Gore** milind_gore@hotmail.com
**Bioinformatics Centre**

After delineation of Chimeric peptide epitopes it is necessary to augment the immune response by encapsulating Chimeric peptides in potent immunogenic form. This can be achieved through microencapsulation in poly lactic acid poly glycolic acid polymers. The work would commence as early as possible.

## 6. Development of polytope DNA vaccine against JE virus

**MM Gore** milind_gore@hotmail.com
**Bioinformatic Centre**

Currently prevalent trends in vaccinology, the epitope-based approach is to prepare a relatively small, but immunologically relevant sequence that is capable of inducing protective immunity against a large and complex pathogen. Chimeric T helper B cell peptide sequences would be incorporated as poly epitope DNA vaccine.

### Objective

To develop DNA polytope vaccine

### Achievements

Attempts to insert the construct using existing endonuclease sites were carried out without success. As an alternative strategy construct was amplified by PCR and cloned into pCDNA 3.1. E.coli transformed with the plasmid were selected on ampicilin and tested for presence of product reactive to JE IPF. Antigen capture ELISA was carried out and two colonies have shown reactivity with Mab Hx-2. Another set of experiments have been planned to insert the construct and express the product to determine its immunogenicty and protective ability.

### Future plan

Reactivty of the product with anti JE serum has confirmed the correctness of designed protein. Both protein and the plasmid would be used for immunization and protection studies.





## 7. Development of a candidate DNA vaccine by prime-boost model for JE virus.

**PS Shah, DA Gadkari** paresh17@hotmail.com
**K. Morita*** *Nagasaki University, Japan

DNA vaccine has been shown to be an effective immunogen in prime-boost model.

As reported earlier, clones expressing PrMEt and NS1 were generated from viral RNA (P20778 Vellore) by RT-PCR using high fidelity Taq Polymerase. The recombinant plasmids were to be tested for their immunogenicity in combination with gold colloid as adjuvant.

### Objective

To determine the immunogenicity and the protective ability of PrM-Et and NS1 genes and the adjuvant effect of gold colloid in mice.

### Achievements

Six weeks old BALB/c mice (n=6) were inoculated with different combinations of recombinant plasmids with/without gold colloid (GC) by i.v. route on days 0 and 9 and 22, to test the immunogenicity. The empty plasmid was included as a negative control and inactivated JE vaccine, procured from CRI, Kasauli, as the positive control. Immunized mice were tested for the presence of anti-JE virus antibodies 10 days after the last dose of immunogen. Sera were assayed by indirect immunofluorescence (IFA), ELISA and *in vitro* neutralization test (NT). Sera from mice inoculated with either PrMEt and/or NS1 genes were positive by IFA indicating that the genes were expressed in vivo and induced an immune response. The highest antibody titres by ELISA as well as NT were observed in mice inoculated with vaccine followed by those inoculated with plasmids PrMEt+NS1. Mice inoculated with either of the plasmids alone showed lower titres of antibodies. Gold colloid did not have an enhancing effect on the antibody titres.

| Immunogen | IFA | ELISA | NT |
|---|---|---|---|
| PrMEt | + | ND | ≤10 |
| NS1 | + | ND | <10 |
| PrMEt + NS1 | + | 250-330 | 10-40 |
| PrMEt (with GC) | + | ND | ≤10 |
| NS1(with GC) | + | 100-220 | <10 |
| PrMEt +NS1(with GC) | + | 250-260 | 10-40 |
| Inactivated JE vaccine | + | 300-400 | 40-160 |
| Empty plasmid | - | <20 | <10 |

In parallel experiments, the recombinant plasmids were also tested for protection against challenge with virulent virus. Three-week-old BALB/c mice were inoculated with different combinations of recombinant plasmids as mentioned above intravenously on day 0 and 9. Twenty-two days after the second dose, mice were challenged with 100 $LD_{50}$ of JE (Bankura)

virus by the i.p. virus / i.c. starch model of infection. No protection was obtained in two experiments.

## Future plan

The DNA vaccine will be used in a prime-boost model to improve the immune response. The boosting antigen will be either baculovirus or SFV particles expressing recombinant protein.





## 8. Identification of neutralization epitopes on the JE virus envelope protein by analysing neutralization escape mutants.

**Cecilia D** cdayaraj@hotmail.com
**JA Pawar**

Epitopes delineated on the E protein can be mapped on the secondary structure of the protein. It could help to understand mechanism of neutralization and design peptide immunogens.

## Objectives

- To determine the association of neutralization with virulence.
- To identify the sites relevant to both the functions on the E protein.

## Introduction

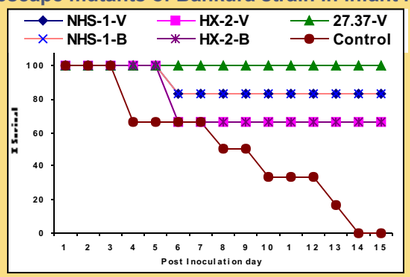
Selection of 15 escape mutants, resistant to neutralisation (NT) by flavivirus cross-reactive HI-positive (HX-2, 27.37) and type-specific non-HI (NHS-1) MAbs had been reported earlier. The mutants had been selected using two strains of JE virus (Vellore and Bankura) and three MAbs.

## Achievements

Mutants representative of each MAb-strain combination were tested for virulence in mice. Infant mice inoculated i.p with the mutants showed longer average survival time as compared to those inoculated with parent viruses (following figures) and complete loss of mortality was observed in adult mice inoculated i.c with the mutants.

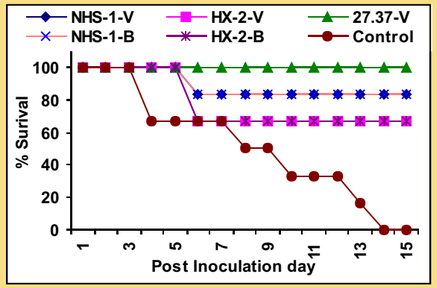
**Virulence of NT escape mutants of Vellore strain in infant mice by i.p. route**

**Virulence of NT escape mutants of Bankura strain in infant mice by i.p. route**



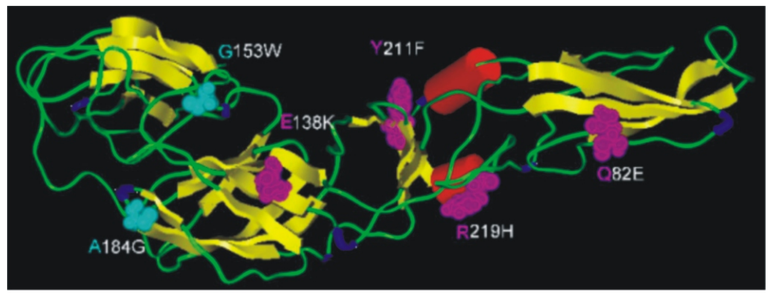
The adult mice, inoculated with mutants were protected when challenged with parent virus indicating that the mutants had replicated to levels sufficient to induce a protective immune response.

**Protection induced by NT escape mutants inoculated i.c. in adult mice**




Sequencing of the E gene of 15 mutants revealed that all mutants of Vellore strain selected with three MAbs had a single nucleotide change leading to a single amino acid change at position 153 (G to W). All mutants of Bankura strain selected with two MAbs had a single nucleotide change resulting in a single amino acid change at position 138 (E to K). Both the amino acids substitutions were major changes and occurred in domains important for the dimerization of E protein, which explained the major changes observed in the mutant phenotype as regards loss of neutralization with several MAbs and loss of virulence. It is important to note that the mutants could still induce a protective immune response.

**Fig.3 The structure of the E protein showing the sites of mutation in the mutants**




## Future plan

Project completed, manuscript in preparation





# 9. Development of attenuated JE virus strain.

**Cecilia D, JA Pawar**
**PS Shah, DA Gadkari**
cdayaraj@hotmail.com

Some of the most successful viral vaccines are live attenuated vaccines.

## Objective

To obtain a stable, avirulent and immunogenic mutant of JE virus.

## Introduction

Three *ts* mutants isolated from the re-established persistently infected cell lines were earlier shown to be avirulent and immunogenic. The mutants were more stable than the parent mutant, *ts* 48 which was used to re-establish persistence.

## Achievements

To establish the stability of the phenotype, two selected mutants were passed twenty times through PS cells and then checked for loss of virulence in mice. Both mutants, 2C (mortality ratio=0/7) and 6H (mortality ratio=0/7) retained lack of virulence in infant mice by i.p. route but only 6H inoculated mice were protected against intracerebral challenge with 100 $LD_{50}$ of parent virus.

## Future plan

The complete genome of *ts* 6H and *ts* 48 mutants will be sequenced and compared to the sequence of the parent Vellore strain to identify determinants of virulence.

## 10. Comparison of the immunogenic potential, antigenicity, stability of inactivated JE virus antigens obtained from *Cx tritaeniorahynchus* with mouse brain derived antigens.

**Roy A** royarbindo@hotmail.com
**Kolhapure RM, Soni GR * NIB NOIDA**

Host induced modifications in the virus occur as found in many viruses when passages are carried out in non-natural but susceptible hosts. Culex mosquitoes are the natural vectors of JEV. It is understood that the antigen obtained from the cell line derived from *Culex tritaeniorhynchus* would have antigens that are normally present in the virus found in nature. Our earlier studies indicated that inactivated JEV antigens obtained from *Culex tritaeniorhynchus* cell line had longer shelf life at 4°C without preservatives compared to the mouse brain derived antigen. The C6/36 cell line was also used and it was found that JEV in its lytic cycle produces lot of UV absorbing material and the antigens thus obtained were not at all stable at 4°C compared to that of *Culex tritaeniorhynchus* cell line.

### Objectives

- The present vaccine (Nakayama) or the standard antigens that are being used in vaccination or for the diagnostic purposes are mouse brain derived. Even the antigen derived from chick embryo fibroblast primary culture contains many host proteins.
- To compare the test antigen derived from mosquito cell line with the standard mouse brain derived antigens by determining Relative Potency with respect to: a) Immunogenicity and antigenicity, b) Stability on storage, c) Presence of UV absorbing contaminants d) Toxicity
- To prepare putative agents for immunological testing and for immunization of animal reservoirs.

### Achievements

Virus grown in *Culex tritaeniorhynchus* cells was inactivated by formalin and the potency of this antigen was tested in 3-4 week old Swiss mice. Different logarithmic dilutions of this antigen were used to immunize the mice with three doses administered on day 0, 3 and 7. The mice were challenged on day 14 or 15 with JE (Nak) virus. This experiment was carried out with 2 batches of antigens, standard antigens and test antigens. However, the relative potency of the test antigens could not be determined since the mice, immunized and control without immunization, were refractory to the virus challenge.

### Future plan

Antigen production in *Culex trotaeniorhynchus* cell line grown on medium supplemented with mulberry and mueri silkworm hemolymph.

Pproduction of protective level of antibodies in larger animals for possible use as veterinary vaccine

Antigen for preparation for diagnostic purpose.





## 11. Studies on immunoprotective mechanisms of JE virus infection in mice

**M.M. Gore** milind_gore@hotmail.com

Understanding of dominant immunoprotective mechanisms and antigens is helpful in vaccine development. Mechanisms of protection from lethal challenge from JE virus are less understood. Using cell transfer and antibody transfer methods dominant immune mechanism and antigens can be studied. The study would be useful in understanding JE pathogenesis.

### Objective

- Animals would be immunized with live or killed virus and challenged with JE virus.
- Immune cells and antisera would be used for passive transfer of immunity in mice and would be assessed for protection.

### Achievements

A model capable of incorporating individual components of immune system involved in protection been developed. JE virus can infect and kill mice by peripheral route till about 12 days of age. Immune cells from adult mice were transferred by iv route in 10 day old mice and then challenged with JE virus by ip route. Mice could be protected from lethal challenge by transfer of immune spleenocytes establishing the model.

Adult mice were immunized with JE virus with 3 and 4 log $LD_{50}$ of JE virus by ip route on 0 and 14th day. Spleenocytes were harvested on the 28th day and infused in 10 day old mice by iv route. Mice were challenged with 2 log of virus by ip route.

**Protection of 10 day old mice passively immunized with Spleenocytes from JE immune mice**

| Immunization dose | Survivors/ Total |
|---|---|
| 100 $LD_{50}$ | 3/4 |
| 1000 $LD_{50}$ | 4/4 |
| Normal Ag | 0/4 |

### Future plan

Experiments are in progress to immunize BALB/c and C3H mice to carry out cell transfer studies at subpopulation and antigen specific level.

## 12. si RNA mediated interference of Japanese encephalitis (JE) virus replication in persistently infected mammalian cells

**PS Shah** paresh17@hotmail.com
**Cecilia D, A Basu, P.S. Sathe, D A Gadkari**

RNA interference (RNAi) is a biological process in which small double stranded RNA (siRNA) fragments result in targeted post-transcriptional gene silencing (PTGS). We had earlier established a porcine kidney (PS) cell line persistently infected (PI) with JE virus. The study was initiated to assess inhibition of JE virus replication in the PI cells by siRNAs.

### Objective

- To cure cells persistently infected (PI) with JEV by targeted delivery of siRNAs.
- To compare the ultrastructural changes in PI cells before and after treatment with siRNA.

### Achievements

Based on the sequence data of JEV RNA three siRNAs, 21 bp long, corresponding to two structural proteins, core and envelope and one non-structural protein, NS5, were designed using Qiagen siRNA designing software. The sequence of the siRNAs were analysed by BLAST for homology with known sequences. No homology was observed. The siRNAs were synthesized with dinucleotide overhangs at their 5' ends (Qiagen).

PI cells, cryopreserved at passage level 102 (P-102) were revived for the curing experiments. The PI cell line was checked for presence of mycoplasma and found to be free by PCR assay. Indirect immunofluorescence assay (IFA) for detecting viral antigen and RT-PCR for detecting viral RNA were standardized to assess the inhibitory effect of siRNAs on PI cells. The IFA test was standardized with mouse hyperimmune sera, which showed strong fluorescence with PI cells.

Two primer pairs targeted to regions in the NS5 (1084bp amplicon) and E (629bp amplicon) genes were used to standardize detection of viral RNA in PI cells by RT-PCR. PI cells from a single well of a 96 well plate (approx. 50,000 cells) gave a positive signal only with the NS5 primer pair. Both primer pairs gave positive signal when the cell concentration was increased 10 fold to 500,000.

### Future plan

The siRNAs will be tested for their ability to inhibit JE virus replication in the PI cells at varying concentrations and in different combinations





## 13. Identification of sites on the E protein relevant to neutralization

**P.S.Shah** paresh17@hotmail.com
**K. Morita*** *Nagasaki University, Japan

(Studies carried out by PS Shah at Institute of Tropical Medicine (IOTM), Nagasaki, Japan as part of the JSPS programme).

Monoclonal antibody 503 is a JE specific monoclonal antibody, which shows high virus neutralization capability. Five amino acids (Envelope - 52,126,136,275 and 367) were shown to be responsible for the epitope. Four amino acids except residue 367 were clustered around a narrow area in the junction of domain I and II. To address the relevance of these positions to various viral functions, genetically engineered variants each with one or multiple amino acid alterations at positions 52 126,136, 275 and 367 were produced.

### Achievements

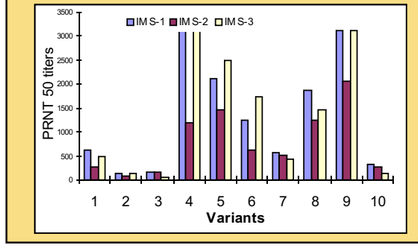
Four variants with a single substitution (#1-4), three with two substitutions (#5-7), one (#10) with four substitutions and two wild type stocks, JaoH0566 (#8) and, JaOArS982 (#9) were analysed for their neutralization profiles with mouse immune sera raised against different Indian strains of JE virus and with sera from humans who were immune either by vaccination or natural infection.

Immune sera were raised against Indian strains Bankura (1973) IMS-1, Goa (1982) IMS-2 and Vellore (1957) IMS-3. The strains were spatio-temporally different and hence differences in their NT epitopes were expected. Of the six different human sera, P-1 and P-2 were from JE vaccinated persons from Kolhapur, P-3 and P-4 were JE survey sera from Andaman and P-5 and P-6 were post epidemic survey sera from Bhivandi.

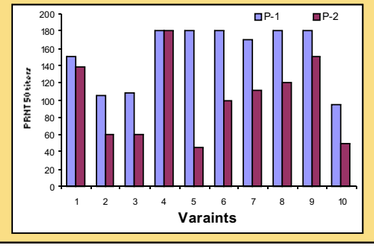
The NT titres of the different sera against the different recombinant viruses were determined by plaque reduction NT test. The histograms show that several recombinant viruses lost NT with mouse immune sera, and sera from humans who were infected with Indian strains. Contrary to that the vaccinee sera did not show such loss in NT activity. This indicated that the epitope defined by MAb 503 was shared by Indian strains, with different mutations important to different strains.

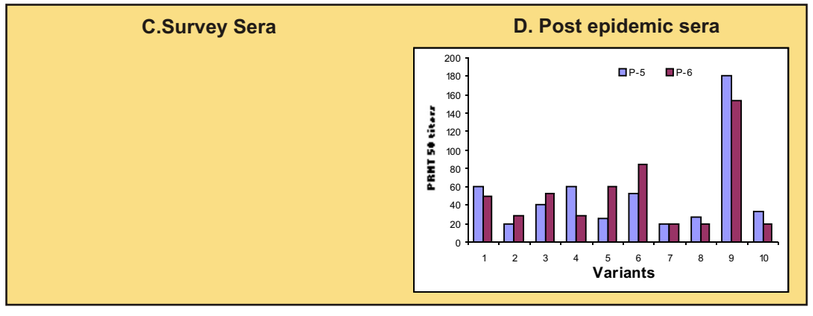
**Plaque reduction Neutralization test of JE variants against immune sera**

A. Mouse immune sera




B. Vaccinee Sera






## Future plan

The manuscript is in preparation.





# 14. Molecular epidemiology of West Nile virus isolates in India.

**V Bondre** vpbondre@hotmail.com

Molecular epidemiology of viruses offers a tool for studying emergence of newer strains and determining the evolutionary relationship between viruses. Genetically WN virus has been differentiated in to two distinct genetic lineages. In India, limited information is available about nucleotide sequences and genetic variations among isolates from different hosts or geographical areas. The study was carried out to characterize the WN virus isolates from different species and human host from Southern India during 1980 and to determine their genetic relationship with strains reported from other countries.

## Objectives

- To characterize WN virus isolates, isolated from different mosquito species and a human host.
- To understand nucleotide sequence divergence in the genomic region-encoding portion of nucleocapsid, complete premembrane and membrane and partial envelope protein of WN virus isolates.

## Achievements

The nucleotide sequence of genomic region encoding portion of C, complete prM/M and partial envelope protein of Indian WN virus isolates and the Egyptian prototype strain E-101 was analyzed and compared with the sequences available in GeneBank. WN virus isolates included in the study were-

1. IND80245Ct (*Cx. tritainiorhynchus* -AY 639639), 2. IND80829Cw (*Cx. whitmorei*-AY 639640),

3. IND80755Cb (*Cx. bitaeniorhynchus*-AY 639641), 4. IND804987Cv (*C. vishnui*-AY 639642),

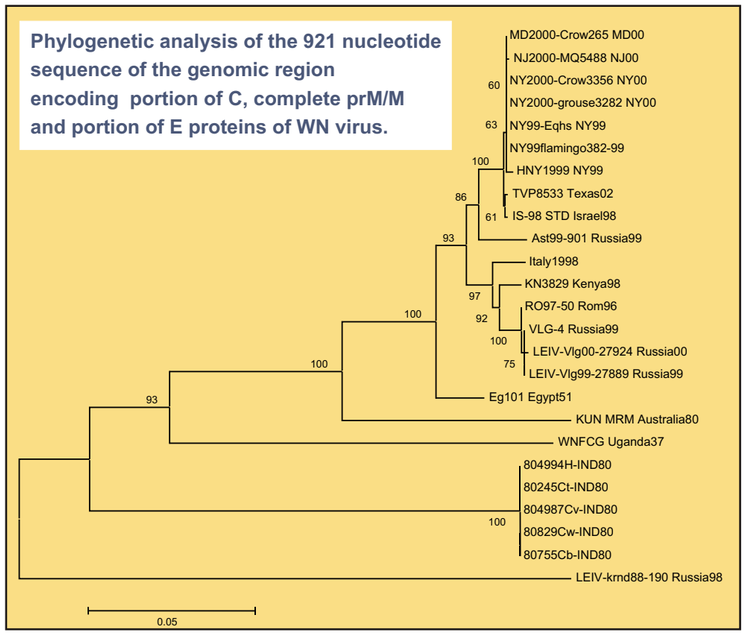
5. IND804994H (Human-AY 639643) and 6. E-101 (Egyptian prototype strain 1951).

Absolute homology in the 921 nt genomic fragment of Indian WN virus isolates was observed independent of the host species. 21% divergence among sequence of Indian and Egyptian strains was observed. Nucleotide sequence alignment alignments with respective sequence of WN virus strains (from other countries) from GenBank shows about 21-26% divergence with lineage 1 and lineage 2 strains including the Australian Kunjin virus suggesting that these strains are substantially divergent at the genomic level. Phylogenetic analysis using the 921 nt sequence as well as smaller fragments encoding portion of C, complete prM and M and portion of E suggests existence of five distinct genotypes / groups among WN virus separated by 21-26%. The WN strains from USA, Israel, Russia, Italy, Egypt, Kenya, etc. comprises the genotype 1; the Australian Kunjin virus comprises the genotype 2; most of the African strains comprise genotype 3; the Indian strains comprise genotype 4 and the avian isolate from Russia LEIV-krnd88-190 comprises the genotype 5. The unrooted phylogenetic tree constructed using different softwares by complete 921 nt sequence as well as shorter fragments encoding portions of capsid, complete premembrane, complete membrane and

portion of envelope protein gave identical pattern indicating that the shorter fragments are also useful in studying the phylogenetic relationship among WN virus.

Indian isolates showed 16 amino acid changes which were not reported among WN virus strains from other countries. Conservation of the amino acid sequence among all five isolates suggests that these are changes independent of host and are characteristics of Indian WN virus isolates isolated during 1980. These unique amino acid changes in the 921 nt genomic fragment will form a marker of identification and can be helpful in understanding the geographical movement of WN virus strains from India.

**Phylogenetic analysis of the 921 nucleotide sequence of the genomic region encoding portion of C, complete prM/M and portion of E proteins of WN virus.**




## Future plan

Genomic and pathogenesis studies will be conducted with other isolates having variable biological properties including virulence in animal models.





# Dengue

## 1. Molecular epidemiology of DEN viruses.

**Cecilia D** cdayaraj@hotmail.com
**PS Shah, JA Pawar**

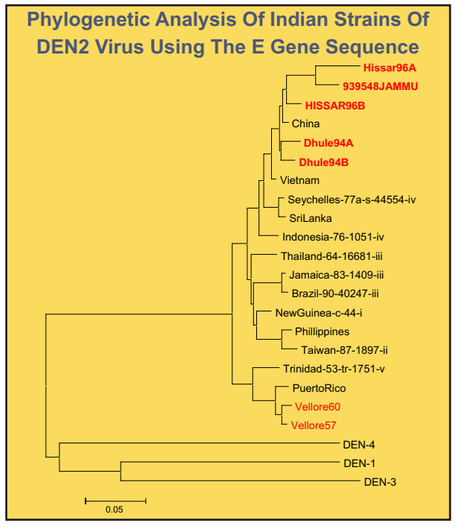
The Asian genotype of DEN2 and genotype III of DEN3 have been incriminated in the occurrence of DHF/DSS. For phylogenetic analysis of dengue viruses, the E gene is reported to yield the best fit clustering. The phylogenetic analysis of dengue 2 viruses based on the partial E gene sequence of 4 strains was reported last year.

### Objectives

Dengue incidence is on the rise and an increase in the incidence of severe manifestations has been observed. Knowledge of the serotypes/genotypes circulating in the country is imperative.

### Achievements

The complete sequence of the E gene (1500 bases) was obtained for 6 strains of DEN2. The strains were obtained from different regions of India Tamil Nadu (1960), Jammu (1993), Haryana (1996) and Maharashtra (1994). The envelope gene sequence was analysed in context to other sequences in the Genbank. The strain isolated from Vellore in 1960 (P23085) showed 98% identity with a 1957 Indian isolate and ~88% identity with 1990 isolates. The five 1990 isolates showed ~96% identity with each other. On phylogenetic analysis, the 1960 isolate grouped with strains from Trinidad and Puerto Rico (genotype V) while the 1990 isolates grouped with Chinese isolates (genotype IV). This indicated a shift in the circulating genotype within the country from the American to the Asian genotype. Whether this finding has any significance in the changing scenario of dengue disease in India will be borne out by sequencing several more strains.




### Future Plans

Sequencing of multiple genes of Dengue viruses is ongoing and full genome sequencing of selected strains will be undertaken.





## 2. Studies on distribution of *Aedes aegypti* in the towns and villages along the western coastal region of India.

**PVM Mahadev** mahadevpvm@yahoo.co.in
**AC Mishra, DT Mourya**

In the light of discontinuous distribution of *Aedes aegypti,* increasing incidence of DEN in rural and urban areas, probable re-emergence of CHIK and possible emergence of yellow fever, present studies were felt necessary in this region.

### Objectives

- Reconnaissance surveys during dry and wet seasons
- Case studies and in depth analyses in representative areas
- Population variation

### Achievements

**Distribution studies of *Aedes aegypti* in Mumbai & Thane District of Maharashtra State (Dry & Wet season survey)**

During dry season a total of 36 localities comprising of 773 households were surveyed in 12 settlements. A total of 30 (83.3%) localities in 9 (75%) city/villages were found positive for *Aedes aegypti*. In the entire survey, a total of 7038 containers were searched out of which 265 were found positive. In the positive settlements, the Breteau index (BI) and Container index (CI) and Adult house index ranged from 12.5 to 57.5, 1.53 to 6.67 and 2.04 to 3.33 respectively.

During the wet season a total of 34 localities comprising of 692 households were surveyed in 12 settlements. Among these 32 (94.1%) localities in all the settlements were positive for *Ae aegypti*. A total of 5155 containers were examined with storage container ratios of 1:6.9 for outdoor: indoor and *Aedes aegypti* positivity ratio of 1.12:1.0 for outdoor: indoor. Among the total water holding containers, 235 containers were found positive for *Aedes aegypti*. BI, CI and AHI ranged from 15.0 to 70.0, 2.14 to 7.84 and 2.5 to 12.0 respectively in the towns positive for *Ae aegypti*.

**_Aedes aegypti_ distribution studies in Saurashtra-Kachchh and South Gujarat region of Gujarat State (Dry and Wet season surveys)**

During the last year, four field trips were undertaken in the Saurashtra-Kachchh and South Gujarat region of Gujarat State. These two regions differ ecologically- Saurashtra-Kachchh region is dry and marshland with forest relicts in Junagadh and Bhavnagar districts; the South Gujarat has sylvan cover extending from the southern border of Valsad to Nadiad district in the north.

During the dry season a total of 12 urban and 10 rural areas consisting of 915 houses in Saurashtra-Kachchh region whereas a total of 8 urban and 4 rural areas consisting of 415 houses in South Gujarat were searched. The prevalence indices in all the positive settlements ranged from BI = 2.5 to 85.0 and AHI = 1.25 to 22.5 in Saurashtra-Kachchh whereas in the South Gujarat the prevalence indices ranged from BI=10.0 to 86.7; AHI=2.5 to 40.0. During the

Water filled tyres on roof tops and arround house holds are preferred breeding areas

Coconut shells, which are used in latex collection in rubber plantation are preferred breading containers for *Aedes* species

Leaf axils of banana, pineapple etc. accumulate rainwater, and support breading of *Aedes* mosquitoes

wet season a total of 13 urban and 8 rural areas consisting of 1214 houses in Saurashtra-Kachchh region whereas a total of 10 urban and 3 rural areas consisting of 694 houses in South Gujarat were searched. In the Saurashtra-Kachchh and South Gujarat regions, 6206 and 4252 water holding containers were searched out of which 541 and 258 containers showed the presence of *Aedes aegypti* respectively. During the wet season the *Aedes aegypti* prevalence increases drastically with positivity in almost all the settlements. The prevalence indices ranged from BI=13.75 to 131.25; AHI=1.25 to 25.0 in Saurashtra-Kachchh and BI=5.0 to 132.35; AHI=5.0 to 41.17 in the South Gujarat. In both the seasons the prevalence indices in South Gujarat were comparatively higher than the indices in Saurashtra-Kachchh. This is probably due to the comparatively faster urban growth and industrial development in the South Gujarat region.

The water storage practices in these regions are different as the number of containers used for water storage in South Gujarat (7.8 containers/house) is more compared to Saurashtra-Kachchh region (5.8 containers/house). In both the regions, similar patterns *Aedes aegypti* breeding were observed, outdoor breeding being significantly higher compared to indoor ($p<0.001$) with RR>3. The *Ae aegypti* showed higher potential of breeding in non-potable containers (RR>6) in both the regions. However, the positivity of the potable containers for *Ae aegypti* breeding cannot be ignored (Saurashtra-Kachchh- Dry (19.6%), South Gujarat- Dry (2.1%). Eight different types of containers were observed in both the regions. The *Ae aegypti* population in both these regions preferred cement tanks ($p<0.001$;RR>4) compared to other types of containers.

Presence of *Ae aegypti* was also recorded in the transportation areas of Gujarat State. During both the dry and wet seasons, a total of 42 transportation areas from Saurashtra-Kachchh (26 in dry; 30 in wet) and South Gujarat (16 in dry; 12 in wet) were searched. These 42 premises consisted of Road transport areas (14 in dry & wet), Rail transport areas (7 in dry; 9 in wet), Seaport (20 in dry; 18 in wet) and Airport (1 in dry & wet). The presence of larval and adult *Ae aegypti* was recorded in Road transport (2 in dry; 10 in wet), Rail transport (2 in dry; 7 in wet) and Seaport (4 in dry; 6 in wet). The spread of *Ae aegypti* by means of transportation ancillaries is well documented worldwide. The larvae of *Ae aegypti* were recorded in tyres in road transport areas and adults were observed in launches and medium sized vessels that travel within and between the states and countries. This poses the risk of further spread of the species.

### *Ae aegypti* and *Ae albopictus* distribution studies in coastal towns/villages in Karnataka and North Kerala State (Dry season surveys)

Dry season: During the dry season a total of 11 urban and 4 rural areas consisting of 604 houses in coastal regions of Karnataka and 4 urban areas consisting of 178 houses in North Kerala were searched. *Aedes aegypti* presence was recorded in 6 urban and 2 rural areas of Karnataka whereas in North Kerala region it was recorded in only one town. *Ae. albopictus* was also recorded in 5 urban and 1 rural area of Karnataka and three towns of North Kerala.





*Ae albopictus* is mainly considered as a tree hole breeder; hence its presence in water storage containers has been a matter of great concern in view of DEN virus dissemination and maintenance.

**Summary of the *Ae. aegypti* & *Ae. albopictus* positivity in the towns / villages in Karnataka and Kerala states**

| Name of the town/village | No. of Houses Searched | No. of containers +ve for *Ae. albopictus* | No. of containers +ve for *Ae. aegypti* |
|---|---|---|---|
| **Karnataka State** | | | |
| Karwar | 65 | 0 | 1 |
| Belkere | 40 | 0 | 4 |
| Gokarna | 32 | 5 | 0 |
| Kumta | 40 | 2 | 0 |
| Honnavar | 32 | 9 | 3 |
| Bhatkal | 44 | 3 | 0 |
| Murdeshwar | 30 | 0 | 0 |
| Mulki | 40 | 1 | 0 |
| Kulur | 36 | 1 | 1 |
| Kochiguda | 33 | 0 | 0 |
| Manglore city | 32 | 0 | 0 |
| Udpi | 67 | 2 | 2 |
| Kundapur | 34 | 0 | 1 |
| Baindur | 40 | 0 | 0 |
| Malpe | 39 | 4 | 0 |
| **Total** | **604** | **27** | **12** |
| **Kerala State** | | | |
| Kasargod | 60 | 0 | 1 |
| Hosdurg | 50 | 1 | 1 |
| Poyanur | 28 | 0 | 0 |
| Kannur city | 40 | 0 | 2 |
| **Total** | **178** | **1** | **4** |

Compact house hold array is of high risk for *Ae aegypti* breeding

## Future plan

The studies will be continued.

## 3. Etiological & epidemiological investigations on dengue virus infections in Karnataka and neighbouring areas.

**P Yergolkar** nivbng@bgl.vsnl.net.in
**K Hanumayya**

Dengue continues to be a major public health problem in Karnataka and neighbouring areas. There is a need to improve early diagnosis for patient management and for undertaking suitable prevention and control measures.

### Objectives

- To diagnose and establish DEN viral infections in suspected patients.
- To study epidemiological features, establish serum/virus bank and data base
- To provide laboratory diagnosis to the concerned physicians for patient management and to the State and District Health authorities for undertaking necessary prevention and control measures.
- Bio-ecological studies on mosquito vectors of and DEN viruses.

### Achievements

**Studies on dengue (DEN) in Karnataka and neighbouring districts of Andhra Pradesh, Tamil Nadu & Kerala.**

During April 2003 to March 2004, blood specimens were received from a total of 3260 suspected cases of dengue. Of these, 2704 were classical dengue fever cases, 242 were DHF/DSS cases (Urban) and 314 cases were classical dengue fever from rural areas. Of the total suspected cases 3225 were from Karnataka including Bangalore city, 20 from Andhra Pradesh, 3 from Tamil Nadu, 1 from Kerala and addresses were not available for 11 cases. There were a total of 217 cases with paired serum specimens and remaining were single serum samples. All specimens were tested for IgM antibodies against dengue-2 antigen in MAC-ELISA.

**Serological diagnosis of suspected dengue cases from Karnataka and neighbouring districts of Andhra Pradesh, Tamil Nadu & Kerala.**

| Sl. No. | District/State | # tested | # Pos. for Dengue | DHF-DSS |
|---|---|---|---|---|
| 1 | Bangalore City - Karnataka | 585 | 326 | 109/133* |
| 2 | Kolar | 499 | 207 | 7/8 |
| 3 | Mandya | 398 | 135 | 6/8 |
| 4 | Bangalore Rural | 322 | 101 | 18/23 |
| 5 | Bangalore Urban | 262 | 94 | 22/29 |
| 6 | Tumkur | 220 | 65 | 3/5 |
| 7 | Mysore | 184 | 55 | 1/1 |
| 8 | Shimoga | 252 | 29 | - |
| 9 | Dakshina Kannada | 65 | 24 | - |
| 10 | Chamarajnagar | 54 | 19 | - |
| 11 | Bellary | 73 | 15 | 3/3 |
| 12 | Davanagere | 27 | 12 | 8/10 |
| 13 | Dharwad | 27 | 11 | 2/2 |
| 14 | Chitradurga | 60 | 10 | 3/3 |
| 15 | Kodagu | 85 | 7 | 0/1 |
| 16 | Bagalkot | 7 | 5 | 2/2 |
| 17 | Belgaum | 13 | 5 | 2/2 |
| 18 | Hassan | 20 | 5 | 2/3 |
| 19 | Raichur | 35 | 5 | - |
| 20 | Bijapur | 9 | 4 | 1/1 |
| 21 | Chikmagalur | 6 | 3 | - |
| 22 | Haveri | 6 | 2 | 0/1 |
| 23 | Gadag | 2 | 1 | - |
| 24 | Gulbarga | 9 | 1 | - |
| 25 | Uttara Kannada | 3 | 1 | - |
| 26 | Koppal | 1 | 1 | - |
| 27 | Bidar | 1 | - | - |
| | Total | 3225 | 1143 | 189/235 |
| 28a | Anantapur Andhra Pradesh | 8 | 3 | 1/1 |
| b | Chittoor | 10 | 1 | 0/3 |
| c | Nellore | 2 | - | - |
| 29 | Tamil Nadu | 3 | 2 | - |
| 30 | Kerala | 1 | - | - |
| 31 | Address not known | 11 | 6 | 2/3 |
| | Total | 3260 | 1155 | 192/242 |

*No.of cases positive for dengue/No. of cases tested

Dengue positive cases were diagnosed throughout the year with maximum cases occurring during May to October with peak number of cases in July as shown in the following table.

**Monthly diagnosis of suspected dengue cases from Karnataka and neighbouring districts of Andhra Pradesh, Tamil Nadu & Kerala, Apr-2003 to Mar-2004.**

| Month | No. of cases tested | No. of cases Pos. for Dengue | DHF-DSS |
|---|---|---|---|
| April - 2003 | 61 | 19 | 3/7* |
| May | 252 | 88 | 5/9 |
| June | 462 | 194 | 31/32 |
| July | 933 | 395 | 56/60 |
| August | 697 | 221 | 39/49 |
| September | 339 | 120 | 31/40 |
| October | 257 | 78 | 14/22 |
| November | 164 | 26 | 8/14 |
| December | 34 | 5 | 1/1 |
| January - 2004 | 29 | 3 | 2/4 |
| February | 10 | 2 | 1/2 |
| March | 22 | 4 | 1/2 |
| Total | 3260 | 1155 | 192/242 |

*No.of cases positive for dengue/No. of cases tested

Out of 3260 cases 1714 were males (642 positives) and 1546 were females (513 positives). Maximum cases and positives were in adults (53.6%), 16.8% were children in <1-5 yrs age group, 19.4% were in the 6-10 yrs, 10% were in 11-15 yrs age group and age was not known in 0.3% of positive cases.

**Age & Sex wise distribution of dengue cases from Karnataka & bordering districts of Andhra Pradesh, Tamil Nadu and Kerala from Apr-2003 to Mar-2004.**

| Age group in years | Male | Female | Total | DHF-DSS |
|---|---|---|---|---|
| < 1 5 | @110/259 | 84/196 | 194/455 | 89/74* |
| 6 10 | 136/313 | 88/252 | 224/565 | 90/69 |
| 11 15 | 77/228 | 38/181 | 115/409 | 29/23 |
| 16 95 | 316/906 | 303/915 | 619/1821 | 33/25 |
| Age not known | 3/8 | 0/2 | 3/10 | 1/1 |
| Total | 642/1714 | 513/1546 | 1155/3260 | 242/192 |

@ No. positive for DEN/total tested.
* No.of cases positive for dengue/No. of cases tested with Clinical DHF/DSS Symptoms

## DHF/DSS

Out of total 3260 suspected dengue cases, 242 had clinical symptoms of DHF/DSS as per the clinical information available in the laboratory request form. Clinical grading of the 242 DHF cases were DHF-I (5), DHF-II (195), DHF-III (27) and DHF IV (15). Dengue diagnosis was confirmed in 192 cases.

Highest number of confirmed DHF/DSS cases were from Bangalore City (n=109) followed by Bangalore Urban (n=22) and Bangalore Rural (n=18). DHF/DSS cases were seen in all months and the activity coincided with the dengue cases reported.

DHF/DSS cases were seen in all age groups, 38.5% in children <1-5 yrs age group, 36% in 6-10 yrs, 12% in 11-15 yrs, 13% in adults (16-47 yrs) and age was not known in 0.5% cases.

### Dengue fever outbreaks in villages of Karnataka

Out of the total 3260 suspected cases of dengue, 525 cases were from 53 villages with dengue fever outbreaks in 8 districts of Karnataka. Out of 525 cases 314 (59.8%) were positive for dengue and 211 were negative for dengue IgM antibodies. District wise details are as follows:

| District | No. of villages | No. ofcases tested | No. of cases Pos. for Dengue IgM | % Positive |
|---|---|---|---|---|
| Kolar | 25 | 199 | 134 | 67.3 |
| Mandya | 10 | 116 | 58 | 50 |
| Bangalore R | 5 | 31 | 20 | 64.5 |
| Bangalore U | 5 | 44 | 26 | 59.1 |
| Tumkur | 4 | 38 | 24 | 63.2 |
| Mysore | 2 | 57 | 29 | 50.9 |
| Chamarajnagar | 1 | 30 | 18 | 60 |
| Chitradurga | 1 | 10 | 5 | 50 |





Dengue fever outbreaks occurred in villages starting from April to October for 1 to 2 months period in all age groups and both sexes. Most of the cases were of classical dengue fever type and two cases of DHF-II (positive for dengue) were hospitalized from Chikkajala village of Bangalore Urban and Kattiganahalli village of Bangalore Rural district. All villages had high indices of *Aedes aegypti* as reported as reported earlier.

## Conclusion

Continued and increased activity of dengue and DHF in Karnataka has been demonstrated by diagnosis of 1155 dengue cases during the period. Increase in the number of dengue cases and outbreaks in villages are being observed.

## Future plan

There is continued need for surveillance and diagnosis of dengue cases for undertaking preventive control measures and better management of DHF/DSS cases.

## 4. Studies on *Ae aegypti* in relation to dengue virus infection in rural areas of Karnataka State, India.

**JP George** nivbng@bgl.vsnl.net.in
**P Yergolkar**

During the previous years epidemics of varying intensities due to DEN virus infection were reported from several villages of many districts of Karnataka State. Therefore it is was thought necessary to study the ecology of *Aedes aegypti,* the main vector of DEN virus in these areas with a view to plan control strategies against this species.

### Objectives

- Pattern of *Aedes aegypti* distribution and seasonal prevalence and dengue virus infection in the villages.
- Factors governing the distribution of *Aedes aegypti* mosquitoes.
- Identification of important breeding sources.
- Susceptibility of the adult as well as larval populations to insecticide.

### Achievements

During the period April 2003 to March 2004, large number of villages in Karnataka State reported febrile illness suspected to be due to dengue virus infection. Entomological investigations were carried out in 76 localities from 11 districts. These villages were from Kolar (30), Bangalore rural (18), Mandya (7), Bangalore urban (7), Kodagu (4), Dakshina Kannada (3), Tumkur & Shimoga (2 each), Chitradurga, Chamrajnagar and Mysore (1 each) districts. Of these 68 villages were found positive for *Aedes aegypti* breeding. The positive villages were from Kolar (7), Chintamani (7), Malur (6), Siddlaghatta and Srinivasapura (3 each), Bangarpet, Gouribidanur and Mulbagal (1 each) taluks of Kolar district; Hosakote (8), Magadi and Nelamangala (3 each), Devanahalli (2), Channapatna & Doddaballapur (1 each) taluks of Bangalore Rural district; Bangalore North (6), Anekal (1) taluks of Bangalore Urban district; Mandya (2), Maddur (2), Nagamangala (1) taluks of Mandya district; Virajpet (2) taluk of Kodagu district; Koratagere (1) taluk of Tumkur district; Sulya (1) taluk of Dakshina Kannada district; Sagara (2) taluk of Shimoga district; Chamarajanagar (1) taluk of Chamarajanagar district, Molkalmuru (1) taluk of Chitradurga district and Tirumakudal-Narasipur (1) taluk of Mysore district.

In these villages 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 *Ae. aegypti* larvae for 100 houses searched), Container Index (CI denotes the percentage of containers positive for *Ae. aegypti* larvae) and Adult House Index (AHI number of houses positive for *Ae. aegypti* adults per 100 houses searched) were recorded.

In the *Ae. aegypti* positive villages the BI ranged from 4.00 to 112.00, CI from 2.25 to 100.00 and AHI from 3.33 to 30.00. Among the 839 positive containers 574 (68.41%) were from Indoor and 265 (31.59%) from outdoor habitats. Cement water storage tanks were the main breeding source and accounted for 77.59% of the total positive containers. Other positive containers were Mud pots (6.32%), Drums (5.48%), Metal (3.69%), old Tyres (3.33%) and miscellaneous containers (3.58%).

During the survey 289 larvae/pupae belonging to 13 species were encountered, 24 from indoor and 265 from outdoor habitats. These include *Ae. vittatus* 84 (29.06%), *Ae. albopictus* 78 (26.99%), *Ae. chrysolineatus* 41 (14.19%), *Ar. subalbatus* 32 (11.07%), *Cx. quinquefasciatus* 21 (7.27%), *Cx. brevipalpis* 8 (2.77%), *Cx. uniformis* 8 (2.77%), *Heizmannia chandi* 5 (1.73%), *An. stephensi* 5 (1.73%), *Ae. gubarnatoris* 2 (0.69%), *Triptroides affinis* 2 (0.69%) & *Ae. pseudotaeniatus* 1 (0.35%) and *Toxorhynchites splendens* 2 (0.69%).

It is interesting to note that few villages in Kodagu (Puliari and Peraji) and Dakshina Kannada (Adimaradaka and Bedarapane) districts, though having confirmed DEN cases, were negative for *Ae.aegypti* mosquitoes. In these villages *Ae. albopictus* was the predominant species encountered during the survey, breeding in rain filled outdoor containers. The DEN vector in these localities needs to be investigated.

A total of 197 *Ae. aegypti* adults consisting of 88 males and 109 females were collected from these villages. A total of 24 pools (11 M & 13 F) consisting of 67 males and 82 females were prepared for virus isolations.

### Conclusion

During the year *Ae. aegypti* mosquito distribution and prvalence were studied in 68 localities in 26 taluks of 11 districts in Karnataka State. The species is being reported for the first time from Chamarajanagar and Kodagu districts of the State.

### Future plan

The study needs to be extended to other areas/districts of Karnataka from where DEN cases/epidemics are being reported.

Vector breeding in association with railways transport : an important risk factor for dengue virus spread

## 5. Isolation of DEN virus from *Aedes aegypti* mosquitoes received from different places in Maharashtra, and Gujarat States India.

**PVM Mahadev** mahadevpvm@yahoo.co.in
**G Geevarghese**

Field-collected mosquitoes from the nosoareas of Maharashtra State are sent to NIV for detection of viral antigen. After identification using IIFT, ELISA, and virus isolation in tissue culture, the results were communicated to the concerned health authorities immediately.

### Objectives

- Detection of DEN virus in mosquitoes from the areas reporting disease outbreaks.
- Mapping the DEN virus activity across the State

### Achievements

Mosquito samples were received from 55 disease outbreaks in Maharashtra State; 10 (18.18%) of these were from urban areas. In all 18 out of 34 districts reported suspected DEN infections. While, in most districts 1-5 episodes were reported, maximum samples came from 17 episodes of disease in Ahmednagar district alone. The samples were comprised of nine species (Table). Five of these areas didn't have *Ae aegypti.* The total mosquito samples consisted of 102 males (46.79%, n=218) and 373 females (51.88%, n=719) *Ae aegypti*. Rural : Urban *Ae aegypti* distribution was 75:27 for males and 316:57 for females. Only female Ae aegypti mosquitoes showed presence of dengue antigen, 11 from rural and 2 from urban areas. In addition a total of 3 males and 57 females were received from Ahmedabad city, Gujarat State, of these 4 females showed presence of DEN virus antigen.

Stored water containers are good breeding containers for *Ae aegypti*

**Species composition of the mosquito samples examined from Maharashtra State**

| Sr. No. | Name of Species | Male | Female |
|---|---|---|---|
| 1 | *Ae aegytpi* | 102 | 373 |
| 2 | *Ae albopictus* | 3 | 5 |
| 3 | *Cx quinquefasciatus* | 63 | 205 |
| 4 | *Cx fuscanus* | 3 | 2 |
| 5 | *Cx tritaeniorhynchus* | 26 | 41 |
| 6 | *An subpictus* | 0 | 17 |
| 7 | *An culicifacies* | 0 | 2 |
| 8 | *An vagus* | 0 | 1 |
| 9 | *Ar subalbatus* | 21 | 73 |
| | Total | 218 | 719 |

### Conclusions

Newer rural areas are showing DEN activity in the districts of Maharashtra. This indicates an extension of existing DEN virus activity.

### Future plan

Monitoring further extension of the virus activity in mosquitoes taking into account the seropositivity rates in the respective districts.





## 6. Studies on molecular aspects of transovarial transmission of dengue virus in *Aedes aegypti* mosquito.

**D T Mourya** mouryadt@vsnl.net
**P Yadav, MD Gokhale, J Bhat, PV Barde**

It is suggested that the probable mechanism of survival of virus during non-epidemic season is the persistence of virus in mosquitoes through TOT since the *Ae. aegypti* eggs can remain alive for several months.

### Objectives

- Monitoring of TOT in *Aedes aegypti* populations in an urban and rural area throughout the year to understand its epidemiological significance.
- To understand environmental influence like high temperature stress on the rate of TOT.

### Achievements

**TOT in dengue-2 inoculated *Aedes aegypti* mosquitoes.**

Earlier, laboratory TOT experiments were carried out on two dengue-2 virus strains viz. Jammu strain [939548] stock prepared in mice and the prototype strain [TR-1751] stock prepared in C6/36 cell lines. Mosquitoes were infected by intrathoracic inoculation with these virus strains. The eggs obtained from the infected female mosquitoes were divided into batches. First batch of eggs were allowed to hatch after 3-4 days of conditioning of eggs. Second and third batches were allowed to hatch after two and three months respectively. Rate of TOT was determined in the F1 progeny by ELISA. Out of 165 pools processed so far, presence of antigen was shown in 18 pools by ELISA. A different set of 103 pools from the same experiments were processed using RT-PCR method. Out of 103 pools processed the presence of virus was detected only in 7 pools.

**Details of the individuals of F1 generations processed for detection of TOT in dengue-2 virus inoculated mosquitoes.**

| Batches infected with Jammu strain of DN-2 | Pools processed by RT-PCR | No. of pools positive | Batches infected with DN-2 of TR-1751 strain | Pools processed by RT-PCR | No. of pools positive |
|---|---|---|---|---|---|
| Stored at RT for 3-4 days | 18 | 2 | Stored at RT for 3-4 days | 18 | 2 |
| Stored at RT for 30 days | 18 | 0 | Stored at RT for 30 days | 13 | 1 |
| Stored at RT for 60 days | 18 | 1 | Stored at RT for 60 days | 18 | 1 |
| Stored at 28°C for 30 days | -- | -- | Stored at 28°C for 30 days | -- | -- |
| Stored at 37°C for 15 days | -- | -- | Stored at 37°C for 15 days | -- | -- |

### Future plan

Work is in progress to determine if dengue virus undergoes any genomic changes while undergoing TOT.

## 7. Study on the identification and expression of certain virus responsive genes in vector mosquitoes.

**DT Mourya** dtmourya@hotmail.com
**P Yadav**

Insects have cellular and humoral mechanisms for defence against micro or macro organisms invading their body. Recent studies have highlighted similarities between pathogen recognition, signalling pathways, and effector mechanisms of innate immunity in Drosophila and mammals, pointing to a common ancestry of these defences. It has been shown that in *Anopheline* mosquitoes the malarial parasite induces a series of expressive products and different immunoresponsive genes. However, in the case of *Aedes*, which are vectors of dengue; nothing is known about the genes responsible for the immune events.

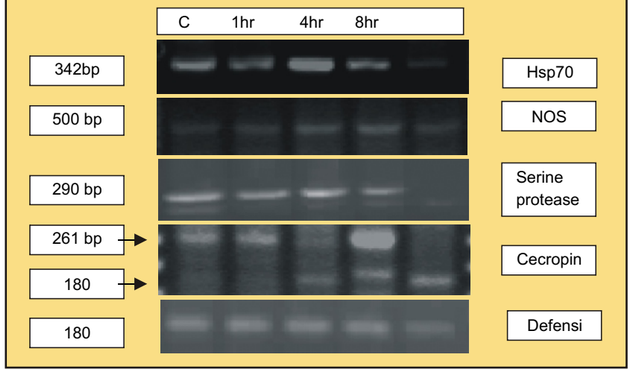
### Objectives

To study the expression of infection-responsive markers and effect of environmental factors on their expression.

### Achievements

*Aedes aegypti* larvae were exposed to 41°C for 60 min and larvae were stored at various time intervals for further processing. Data suggest that expression of certain IR genes was affected due to heat shock. Some of these genes were up-regulated while some were down-regulated. Hsp70 showed the highest expression levels at 4th post heat shock hour (PHSH); NOS was expressed at all time points but higher levels at 4 & 8 PHSH. Serine protease showed lowest expression level at 24th PHSH. Cecropin 180 bp is expressed exclusively on 4th, 8th & 24th PHSH in larvae. Defensin expression was not affected by heat shock.

**Expression profiles of Hsp70, NOS, Serine protease, Cecropin and Defensin in 4th instars larvae**




### Future plan

Project is concluded.





## 8. Development of monoclonal antibodies against dengue virus (2, 3 and 4 serotype).

**DT Mourya** dtmourya@hotmail.com

The laboratory diagnosis of dengue virus infection is established through serological techniques like ELISA, IgM capture, virus isolation and PCR. The need for developing diagnostic monoclonal antibodies specific to dengue serotypes is essential for rapid virus diagnosis.

### Objective

To develop monoclonal antibodies against dengue virus.

### Achievements

Monoclonal antibodies were generated against the three serotypes of dengue viruses using standard protocol. After first and second screening a total number of 11 clones were obtained, out of these 2 were found reacting against only dengue viruses. None of the clone showed any serotype specificity.

| Clones raised against | DEN 1 | DEN 2 | DEN 3 | DEN 4 | JE | WN | Normal mouse brain |
|---|---|---|---|---|---|---|---|
| 157 (D2) | +++ | +++ | +++ | - | - | - | - |
| **19A (D3)** | +++ | +++ | +++ | +++ | ++ | + | + |
| **21A (D3)** | +++ | +++ | +++ | +++ | ++ | + | + |
| 101 (D3) | +++ | +++ | +++ | +++ | + | - | - |
| **141A (D3)** | +++ | +++ | +++ | +++ | ++ | + | + |
| 241 (D3) | +++ | +++ | +++ | +++ | - | - | - |
| 266 (D3) | +++ | +++ | +++ | +++ | + | - | - |
| 110 (D4) | ++ | ++ | ++ | + | - | - | - |
| 128 (D4) | +++ | ++ | ++ | ++ | - | - | - |
| 232 (D4) | +++ | +++ | +++ | +++ | ++ | + | + |

### Future plan

No serotype specific clone was obtained for these three serotypes. Attempts are being made to establish MAbs for DEN-1 serotype.

Surveillance of DEN virus activity in the wild caught immature and adult Aedes aegypti mosquitoes in the Western Coastal region.

Rainfall pattern along with increase in breeding habitats of Aedes mosquitoes can be correlated as a predictive indicator of DEN transmission. It is argued that TOT does contribute in initial virus amplification cycle and can be gauged by detection of dengue virus in Aedes larvae.

Fishing boats with potable water in Gujarat and Maharashtra support *Ae aegypti* breeding

## 9. Surveillance of DEN virus activity in the wild caught immature and adult *Aedes aegypti* mosquitoes in the Western Coastal region.

**PVM Mahadev** mahadevpvm@yahoo.co.in

It is argued that TOT contributes in initial virus amplification cycle. This can be gauged by detection of dengue virus in *Aedes* larvae which can inturn be used as a survillance tool.

### Objectives

To develop and employ a method to preserve mosquito larvae during transport to laboratory for detection of dengue virus antigen.

### Achievements

An aqueous solution of sodium azide (500ug/ml) was useful for the storage and transportation of field collected mosquito larvae and pupae and subsequent detection and isolation of DEN viruses from field collected samples. Detection of dengue viral antigen was carried out by antigen capture ELISA and virus isolation was attempted from larval lysates. DEN virus antigen was successfully detected at Tuna, Jamnagar, Harshad, Vadodara and Daman.

### Rationale and methodology.

Larvae putrefied as soon as they die due to bacterial contamination. Easiest way to arrest bacterial growth is by adding sodium azide. Sodium azide acts on the electron transport chain and thus does not affect the virus as there is no respiratory enzyme system in virus. Removal of azide from larvae is easy by washing in saline. This method was employed and tried in the laboratory and it was found that 500 g/ml of sodium azide can be used to store larvae at room temperature for nearly 15 days. This method was employed for transporting Aedes larvae collected during survey of Gujarat coast. Detection of dengue viral antigen was carried out by antigen capture ELISA and virus isolation was attempted from larval lysates. These larvae were stored in 70% ethanol for identification. A separate pool consisting of 10 to 25 live larvae were stored in aqueous solution of NaN3 (500 g/ml) and brought to laboratory at ambient temperatures 22-32$^{0}$ C for virus antigen detection. Laval samples were processed for detection of antigen in antigen capture ELISA by using HX-2 (Flavivirus cross reactive antibody) for capture and biotinylated Hx-2 for detection. Larave were soaked in PBS for 30 min washed once and then sonicated in PBS containing 1mM PMSF.

### Gujarat

The mosquito surveys were conducted during April-may (Dry) and July-August 2003 (Monsoon) using one larva/pupa per container technique to assess the relative abundance of the vector and larval pools for detection of DEN virus. In all 38 out of 103 pools (581 out of 1336 larvae) were tested DEN positive. The minimum infection rate was 1:37. A total of 460 adults mosquitoes tested in 27 pools were found negative. Further verifications of these results is in progress.





The positive pools originated at the port towns of Tuna, Jamnagar, Harshad and Daman. Of these first three showed DEN Presence during dry and wet seasons. In addition larvae collected in a tyre dump in the Vadodara city also showed the presence of DEN virus antigen.

### DEN activity at Daman

An outbreak of dengue, the first ever, was recorded in Daman (U.T.) in July -August 2003. Attempts were made to determine TOT in the vector mosquito species. The sodium azide preservation method was employed. Pools of 10 to 25 live larvae preserved in sodium azide (500 g/ml) solution were brought to laboratory for virus detection.

A total of 11 larval pools (n=143 Aedes larvae) were collected consisting of six *Aedes aegypti*, four *Aedes albopictus* and one *Aedes vittatus* were processed by ELISA using Hx-2 Flavivirus cross reactive antibody. A total of 5 pools consisting one *Aedes aegypti*, three *Aedes albopictus* and one *Aedes vittatus* showed the presence of DEN virus antigen. DEN activity in mosquito larvae during a concurrent epidemic is interesting. Further verifications of these results is in progress.

Cement tanks : breeding habitat for *Ae aegypti* in Gujarath

# 1. Prevalence of Chikungunya virus infection in India.

JP Thakare jyothakare@hotmail.com
DT Mourya, S Hundekar

Chikungunya (CHIK) virus infection many a times resembles dengue infection and both have a comman vector i.e. *Aedes aegypti* mosquitoes. Virus etiology is evident in hardly 20% of the pyrexia of unknown origin (PUO) or dengue cases. It is logical to screen the PUO and dengue cases for antibodies to CHIK virus. With this rationale we started this work in 2001.

## Objectives

Retrospective and prospective study on the prevalence of CHIK virus by means MAC ELISA and by HI test in DEN endemic as well as non-endemic areas.

## Achievements

Monoclonal antibody (MAb) based MAC ELISA to establish recent virus infections from human samples was standardized incorporating samples collected during CHIK virus epidemic at Barsi, 1973. MAb based antigen capture ELISA to detect virus from field-collected mosquitoes was also standardized. These two techniques were employed for further studies.
To study the activity of virus infection in population, 865 samples collected from fever cases during a period of 2001-2004 mainly from Maharashtra, Gujarat, Kerala and Andhra Pradesh were tested in MAC ELISA. Evidence of recent infection to CHIK virus was established in 32 samples. The true positivity of IgM results was confirmed by 2-mercaptoethanol treatment. Representative samples from these areas were screened by Haemagglutination Inhibition (HI) test. Only 12/101 samples were positive in HI test. In 8/12 of the HI positive samoles, titres were 10, three samples had HI titre 20 while only one sample had a titre of 40.

**Activity of Chikungunya virus 2001-2004**

| Place | No positive | No tested | % positive |
|---|---|---|---|
| Gujarat (Surat) | 1 | 116 | 0.86 |
| Kerala | 7 | 94 | 7.45 |
| Hyderabad (AP) | 3 | 47 | 6.38 |
| Maharashtra | | | |
| Nadurbar | 0 | 5 | 0.00 |
| Dhule | 0 | 6 | 0.00 |
| Nanded | 1 | 21 | 4.76 |
| Aurangabad | 0 | 21 | 0.00 |
| Vidarbha | 11 | 212 | 5.19 |
| Pimpri-Chinchwad | 3 | 82 | 3.66 |
| Kolhapur | 5 | 153 | 3.27 |
| Sholapur | 1 | 70 | 1.43 |
| Satara | 0 | 11 | 0.00 |
| Bhor | 0 | 13 | 0.00 |
| Raigad | 0 | 10 | 0.00 |
| Sindhudurg | 0 | 4 | 0.00 |
| Total | 32 | 865 | 3.70 |

Neutralization test (NT) was carried out by 50% CPE inhibition method. Neutralizing type of antibodies was detected in 67% of the samples collected during Barsi epidemic. Interesingly the samples collected during a period of 2000-2004, which were positive by MAC and or HI test, were not neurtrlizing type. The results support the presence of CHIK virus activity in India Maharashtra and Gujarat.

## Future plan

Study will be continued by means of molecular biology approach.





# Chikungunya

# Hepatitis

## 1. Development of candidate vaccine for hepatitis E.

**TM Deshmukh** varankalle@yahoo.com
**KS Lole, VA Arankalle**

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 a considerable mortality among pregnant women. In sporadic settings, fulminant hepatitis E has been observed in men and non-pregnant women. Infection among travellers to endemic countries is common. Hepatitis E outbreaks have been shown in military establishments. Sewage workers are at a high-risk of Hepatitis E virus (HEV) infection. Hepatitis E among HBV and HCV carriers may have serious complications. Hence there is a need for hepatitis E vaccine.

### Objectives

To develop: (1) Recombinant protein-based (2) Viral DNA-based and (3) Prime and boost approach based hepatitis E vaccine candidates.

### Achievements

Balb/c (inbred strain) and Swiss albino (outbred strain) mice were immunized with ORF2 and GM-CSF plasmids together or ORF2 plasmid alone. Two routes were used for immunization. Intramuscular (im, needle injection, 4 doses of 100g plasmid / dose) and intradermal (id, gene gun, 3 doses of 0.01, 0.1, 1 and 2 g plasmid / dose). Mice were bled at intervals and monitored for the appearance of specific antibodies in ELISA. In another experiment mice were primed with DNA (gene gun, 1g / dose / plasmid) and boosted with 2 g recombinant ORF2 protein (im). Late seroconversion was observed with the needle injection method, anti-HEV titers were 1: 10 while control mice remained negative (data not shown).

Following graph illustrates antibody profile recorded from 0-105 days post dose one of each Swiss albino mouse from group V that was given ORF2 plasmid. Early seroconversion was observed with the gene gun method.

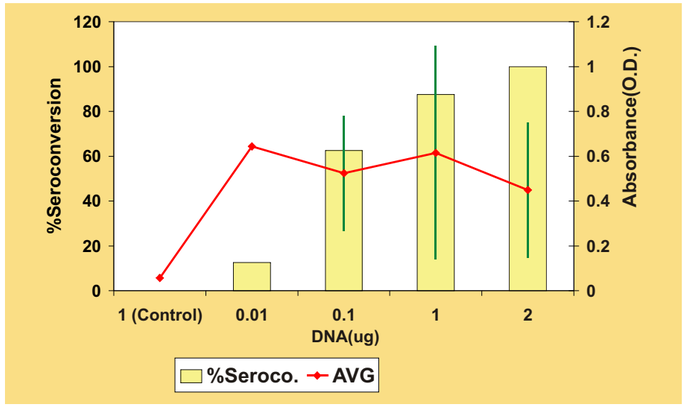


(Absorbance in ELISA monitered upto 15 weeks post dose 1 in group V Swiss albino mice (n=8?, mice ID SAM33-SAM40) receiving pcDNA3.1 + ORF2 (2g/dose, gene gun). Dose schedule ~ 0,4 & 8 weeks. Serum dilution 1:10.)





Following graph shows the extent of seroconversion with each id dose of ORF2.




(Percent seroconversion at 5th week post dose 1. Secondary Y-axis indicates mean ± SD absorbance values at same time point for groups receiving 1, 0.01, 0.1, 1 & 2g/dose/plasmid {I, II, III, IV & V respectively}of Swiss albino mice.)

Mice from control group remained negative. The optimum dose for gene gun method was 1g DNA. However 10ng dose also resulted in seroconversion. Following graph illustrates antibody profile recorded from 0-140 days post dose of individual Balb/c mouse from group B that was given ORF2 plasmid.

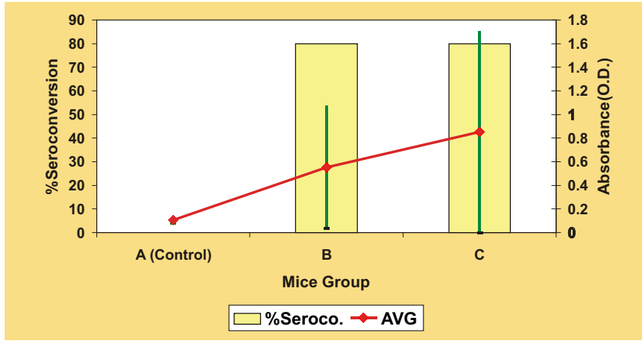


(Balb/c mice, n=5, receiving ORF2 plasmid, 1$\mu$g/dose, id, with dose schedule 0, 2, 4 & 6 weeks. rORF2p + CFA were given intramuscularly as fourth dose. Serum dilution 1:10.)

Following graph illustrates antibody profile recorded from 0-140 days post dose of individual Balb/c mouse from group C that was given ORF2 and GMCSF plasmids. Antibody titers were relatively higher in mice receiving GM-CSF plasmid.

(Balb/c mice, n=5, ORF2 + GM-CSF, 1$\mu$g/dose, id, with dose schedule 0, 2, 4 & 6 weeks. rORF2p + CFA was given intramuscularly as fourth dose. Serum dilution 1:10)

To see the DNA prime and protein boost effect, mice from groups B & C were boosted with recombinant ORF2 protein. Boosting effect of rORF2p was observed within 2 weeks with hundred folds increase in the antibody titer. Mice from group A (control) remained negative. Taken together the results at 5$^{th}$ week post dose the profile was as follows:




(Percent sero conversion observed at 5$^{th}$ week post dose 1. Secondary Y-axis indicates mean ± SD absorbance values at same time point for groups A, B & C of Balb/c mice)





## 2. Cloning and sequencing of the entire genome of swine HEV and expression of ORF-2 & ORF-3 proteins

**LP Chobe** leenachobe@hotmail.com
**KS Lole, VA Arankalle**

Zoonotic spread of HEV has been shown in different countries. Our earlier studies have shown circulation of different genotypes in humans (Type I, 1976-2003) and pigs (type IV, 1985-2000). Type I HEV could not be transmitted to pigs and rats. In order to understand the role of swine HEV in causing human infections it is necessary to express and assess immunoreactivity of swine HEV proteins. Genomic characterization of swine HEV in circulation is of utmost importance.

### Objectives

- To clone and sequence the entire genome of swine HEV
- To express ORF-2 and ORF-3 proteins and evaluate immunoreactivity
- To use the sequence information for the generation of full-genome cDNA clone.

### Achievements

The Indian pig HEV isolate (Ind-sw-00-1) has genomic length of 7240 nucleotides excluding poly (A) tract at 3' terminus and possesses three major ORFs. ORF1 is 5121 nt in length and encodes product of 1707 aa. ORF2 comprises 2022 nt and encodes 674 aa. ORF3 is 342 nt and encodes a polypeptide of 114 aa. Strategy for translation of ORF2 and ORF3 is similar to the one reported for genotype IV human and swine isolates.

The Indian swine HEV isolate has an insertion of a single nucleotide (C) at position 5159 that affects both ORF2 and ORF3 translations. The 5'NTR comprises of 25 nucleotides and shows high degree of conservation with other HEV isolates. The 3'NTR comprises of 70 bases excluding the poly A tail and shows considerable nucleotide diversity compared to other HEV strains. Similar to other HEV isolates a hypervariable region of unknown function was identified between aa 680-796, showing only 74.3% identity with prototype IV. The ORF2 is 42 nucleotides longer than that of other isolates of genotype I-III. Within genotype IV, the percent identity in this region was higher at amino acid level than at nucleotide level, as the changes mostly occurred in the third position of the codons. In ORF2 region, at 5' and 3' ends, the amino acid sequence for Indian swine HEV isolate varied considerably as compared to the other isolates (Type I-IV), but remained highly conserved between a.a.136-490. As compared to type IV prototype, 21 amino acid changes were recorded over the entire ORF2. A hydrophobic signal peptide was identified at the extreme amino terminus of ORF2 protein using a signal peptide prediction program. Twelve changes in amino acids were recorded in ORF3 region as compared to prototype IV.

### Comparison of type IV and type I recombinant ORF2 protein based ELISAs

ELISAs based on recombinant antigens derived from human HEV and swine HEV were compared using different sets of serum samples. Recombinant ORF2 antigen derived from

swine hepatitis E virus (genotype IV) and human hepatitis E virus (genotype-I) differ by approximately 9% at amino acid level.

### Experimental animals infected with genotypes-I and IV

Serial serum samples of rhesus monkey (MM1), experimentally infected with human HEV strain (Akl-90, genotype I) and a pig, experimentally infected with swine HEV strain (genotype IV) were tested for IgG-anti-HEV antibodies in ELISA using both the antigens. Very similar values were obtained, regardless of type of antigen. Pearson's correlation coefficient (r) between the two tests was found highly significant (r=0.975 for pig samples and r=0.943 for monkey samples).

**A: Comparison of Human & Swine Antigen for Monkey Sequential Samples**



**B: Comparison of Human & Swine Antigen for Pig Sequential Samples**



*Comparison of human and swine HEV antigens for detecting anti-HEV antibodies in sequential serum samples collected from experimentally infected (A) monkey, genotype-I and (B) pig, genotype-IV (S/Co=sample OD / cut off value).*

### IgM-anti-HEV detection

A total of 316 patients, bled 1-13 weeks post-onset of clinical symptoms during waterborne epidemics, were tested for the presence of IgM-anti-HEV antibodies using both antigens. A 97.7% (r = 0.718, = 0.612) concordance was observed.

In order to assess false positivity of IgM-ELISA, serum samples from 180 school children, the age group in which the disease is known to be less prevalent, were screened. None of themwere positive with human HEV-ORF2 and one was positive with swine HEV-ORF2, (r=. 701, =. 799) concordance, documenting that both the tests were highly specific.

### Detection of IgG-anti-HEV

The same samples were screened for the presence of IgG-anti-HEV antibodies. In the patient category, 96.8% (r = 0. 896, = 0. 890) concordance was noted, 10/316 samples giving discrepant results, showed borderline OD values. AntiHEV IgG positivity among school children was 296/316 with human HEV and 294/316 (swine HEV) with 96.8% (r =0. 896, =0. 890) concordance.

### Comparison with commercial kit (Gene Lab, Singapore)

Randomly selected 263 serum samples from patients were tested for anti- HEV-IgM using a commercially available kit (Gene Lab, Singapore). Positivity was 187/263 with Gene Lab, 222/263 with human-ORF-2 and 221/263 with swine-ORF-2. The same 35 samples negative by Gene Lab ELISA were scored positive by both the human and swine HEV-based ELISAs. Another seven samples showed discrepant results with respect to different ELISAs.

IgG-anti-HEV positivity was 204/243 with Gene Lab, 222/243 with human-ORF-2 and 223/243 with swine-ORF-2. The same 18 samples were positive by NIV ELISAs and negative by Gene Lab ELISA. Nine samples showed discrepant results with respect to different ELISAs.

## Conclusion

ELISA based on swine HEV-ORF$_2$ was comparable with human HEV -ORF$_2$ based ELISA. The project is completed.

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

**KS Lole** lolekavita37@yahoo.com
**VA Arankalle**

Though difficult, generation of infectious cDNA clones has proved useful in the study of host/virus interactions and mechanisms of viral replication. In the absence of an *in-vitro* cell culture system generation of infectious cDNA will prove useful for basic studies on HEV. Mechanism of species specificity of HEV could be understood.

### Objectives

- To generate infectious cDNA clone for swine HEV and human HEV
- To generate chimera of swine & human HEV.

### Achievement

Complete genome of swine HEV cloned in pGEM-T-Easy vector (TA cloning vector) was subcloned in pGEM-T-Easy vector for introducing T7 promoter for the transcription. *In vitro* transcription of complete viral genome is being standardized.

### Future plan

Once complete genome length RNA is obtained it will be used further for transfection in different cell lines to produce infectious particles.





## 4. Assessment of host/virus factors leading to fulminant hepatitis E and A

**AS Tripathy** anuradhastripathy@hotmail.com
**MS Chadha, VA Arankalle**

Our earlier studies have clearly shown that in sporadic settings, HEV and HAV are mainly responsible for fulminant hepatic failure (FHF) among adults and children respectively. High mortality among pregnant women remains the characteristic feature of HEV epidemics, though a significant proportion of pregnant women also experience subclinical infection. Risk factors for development of FHF are yet to be ascertained. Study of mechanism of FHF may lead to development of appropriate treatment protocols and identification of prognostic markers.

### Objectives

To study the mechanism(s) of fulminant hepatic failure following hepatitis E and A infections

### Achievements

For Hepatitis A, the cytokine study was extended from serum to cultured PBMCs. Blood samples from ten children suffering from self-limiting acute hepatitis A were obtained during epidemics of hepatitis A in Chiplun and Thathwade in Maharastra. Both the Th1 (IL-2, IFN-ã) and Th2 (IL-4, IL-10) cytokine levels were estimated in the supernatants of mitogen stimulated (Phytohaemagglutinin-P, PHA-P + Phorbol 12-myristate13-acetate, PMA) supernatants of lymphocyte cultures using ELISA (ELISAs) (ELISA SETS, BD Pharmingen, San Diago, USA). In HAV patients, IL-2 levels were significantly elevated when compared with IL-4 levels. IFN-ã levels were highly significant when compared with IL-10 levels. Levels of significance of cytokines were determined using Student's t-test.

**Cytokine levels in self-limiting hepatitis A**

| | Serum (n=32) (pg/ml) | PBMCs supernatants (n=10) (pg/ml) |
|---|---|---|
| IFN $\gamma$ vs IL 10 | 106 ± 197 vs 54 ± 113 ($p<0.001$) | 184 ± 313 vs 12 ± 14 ($p<0.001$) |
| IL 2 vs IL 4 | 91 ± 233 vs 34 ± 72 $p < 0.01$ | 128 ± 123 vs 15 ± 10 $p < 0.01$ |

In controls, mean: IL2 (<7.8), IL4 (<4.6), IL10 (21.5) and IFN (13.9) pg/ml

In our study, all the acute resolving HAV patients (n=32, serum-based assay; n=10, cultured PBMCs) universally and significantly released IFN-$\gamma$ suggesting its possible involvement in the elimination of HAV.

## 5. Determination of occupational risk of Hepatitis E Virus in Animal handlers, Sewage Treatment Plant Workers, Water Treatment Plant Workers and Safai Kamgars from Corporations.

**MS Chadha** mscniv@hotmail.com
**CS Raut, SR Vaidya, Arankalle VA**

Recent data generated in several countries suggest zoonotic spread of HEV. In developing countries with inadequate sanitation and overburdened public health infrastructures, more than 50% of sporadic cases of viral hepatitis can be attributed to HEV. The predominant mode of HEV transmission is by fecal-oral route. Hence, sewage treatment plant workers, water treatment plant workers and safai kamgars from corporations may be at increased risk for HEV.

To study the role of animals in the spread of hepatitis E among humans, it would be important to evaluate the risk of animal handling in exposure to this virus. Similarly, other high-risk populations need to be studied to evolve definite preventive strategies, if necessary.

### Objectives

- Determination of occupational risk of HEV infection among animal handlers, sewage treatment Plant workers, water treatment plant workers, safai kamgars from Pune Municipal and Pimpri Chinchwad Municipal Corporations.
- To look for different HEV genotypes in acute cases of hepatitis among animal handlers.

### Achievements

To determine the involvement of animals in transmission of HEV, exposure of animal handlers to HEV was studied. Blood samples of animal handlers including veterinary doctors and cattle abattoir workers after recording a questionnaire.

Seventy six percent (26/34) and 67.4% (31/46) of the doctors and cattle slaughters respectively were IgG HEV positive. This was significantly higher exposure as compared to age matched urban population.

### Future plan

Further prevalance studies will be carried out





## 6. Assessment of role of HCV HVR1 and host HLA status in influencing progression of hepatitis C and response to antiviral therapy.

**S P Shrotri** varankalle@yahoo.com
**A S Tripathy, M S Chadha, V A Arankalle**

Hepatitis C virus (HCV) is an important cause of chronic liver diseases worldwide. There are six major genotypes of HCV exhibiting a high degree of genetic heterogeneity. The greatest degree of heterogeneity is observed in the N terminal region of the envelope region (E2) and is known as the hypervariable region 1 (HVR1). Evolution and changes in this region have been shown to be associated with the outcome of HCV infection and success of antiviral therapy. Further, HLA alleles of the patient may influence the severity of disease and response to interferon therapy.

### Objectives

To assess the role of :

- HVR1 heterogeneity
- HLA status of the host

in relation to disease progression and success of antiviral therapy.

### Achievements

**Sample Collection**

A total of 45 samples were subjected to HVR1 amplification out of which 31 were amplified [genotypes 1a (5/5), 1b (8/8), 1c (0/2), 3a (12/15), 3b (2/9), 3g (5/7), 3i (4/4)]. PCR product sequencing for 23 samples was completed. 31 HVR1 PCR products were TA-cloned into pGEM-T Easy Vector and sequencing of 20 clones each of 24 of the cloned samples was completed.

Thirty-one non-consanguis anti-HCV positive individuals from different parts of Maharashtra and 67 ethnically matched healthy controls were examined. Third generation enzyme linked immunosorbent assay was used for the detection of anti-HCV antibodies. Genomic DNA was extracted from frozen peripheral blood mononuclear cells and the HLA typing was done by PCR-sequence specific primer low resolution (PCR-SSP) method. The significant findings were (i) antiHCV positive Maharashtrians revealed a significantly higher frequency of HLA A*03 (OR, 14.51, EF, 0.4, p=0), A*32 (OR, 1742, EF 0.23, p=0) and HLA B*15 (OR, 13.46, EF 0.38, p=0) when compared with the controls. (ii) Haplotype DRB1*11-DQB1*03 was significantly higher among HCV infected individuals when compared with the controls (OR, 5.86 HF, 19.56, p=0.0001). It seems that HLA class I and class II alleles play vital roles in disease association.

## 7. Intra familial spread of hepatitis C

**M S Chadha** mscniv@hotmail.com

The extent of intra familial spread of HCV in India is not known. Immuno-compromised patients are known to circulate HCV in high titres. For contact management, a comparison of HCV spread among contacts of healthy carriers and immuno-compromised patients would be necessary.

### Objectives

- To determine extent of HCV infection among families of Hepatitis C patients/ carriers/ immuno-compromised patients.
- To understand routes and risk factors of transmission.

### Achievements

As no anti-HCV positive carriers could be identified, family contacts could not be studied. Family contacts of HCV-infected patients on haemodialysis are being examined.





## 8. Genomic characterization of hepatitis A virus isolates recovered from Pune.

**S D Chitambar** Chitambars@hotmail.com
**K S Lole, M S Joshi**

Hepatitis A virus poses a major public health problem in India. Assessment of prevalence and distribution of hepatitis A virus variants in different geographic regions of world is of epidemiological importance. Information regarding the circulating HAV genotypes and their prevalence in Indian population is under reported in spite of its hyper endemicity.

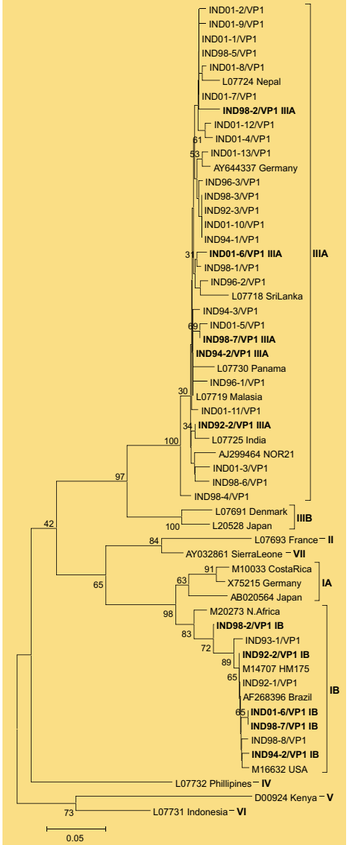
### Objectives

To sequence variable (VP1/2A) and conserved (RNA polymerase) genomic regions of HAV isolates from Pune. To determine the genotypes and variations in HAV strains circulating in Pune by analysis and comparison of nucleotide sequence data.

### Achievements

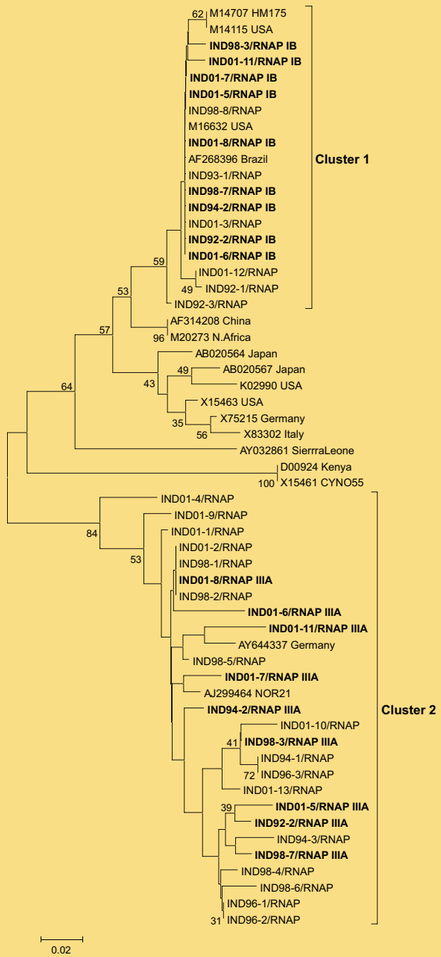
The molecular epidemiology of HAV was studied by investigating stool/serum samples collected from sporadic cases of acute hepatitis A over a period of 10 years. HAV RNA was detected by RT-PCR in 31 of 34 specimens seropositive for IgM anti-HAV. The HAV genomes present in the 31 specimens were examined by sequencing products of VP1/2A junction (168 bp) and RNA polymerase (116 bp) regions. Extensive heterogeneity among HAV strains recovered from hepatitis A patients was evidenced by presence of (i) subgenotype IB and IIIA, (ii) their mixed infection in individual hepatitis A patients and (iii) by diversity in RNA polymerase region in HAV isolates of genotype IIIA investigated in this study.

**Phylogenetic analysis of VP1/2A region**




The isolates in bold letters indicate mixed infections.

**Phylogenetic analysis of RNAP region**




The isolates in bold letters indicate mixed infections.

## Future plan :

The above project will be extended for genetic characterization of HAV isolates obtained in an outbreak of hepatitis A that was detected in children from a school located in a state of Kerala.





# 9. In vitro studies on growth and characterization of Indian isolates of Hepatitis A virus

**R S Fadnis** rahul_fadnis@yahoo.co.in
**S D Chitambar**

Hepatitis A virus (HAV) infection in India has been highly endemic. However, no reports are available on HAV strains recovered from Indian patients with hepatitis A.

## Objectives

To isolate HAV strains in tissue culture system and characterize them

## Achievements

I. Rapid passaging of BGMK cell line infected with HAV strain NIVIN97 reported previously was carried out by reducing the period between sub cultures from 12 days to 3 days and rapidly replicating strain was thus selected. Presence of this virus was detected by ELISA. This strain of HAV was also inoculated in MRC-5 cell line and is being serially passaged.

On the basis of nucleotide sequence of VP1 / 2A junction region (168 bps), NIVIN 97 strain of HAV was characterized to be genotype IB. Full length nucleotide sequencing of this strain at passage level 28 was undertaken. The primers were designed on the basis of prototype strain HM 175 and synthesized. Cell lysate of BGMK cells with HAV was sonicated and RNA was extracted.cDNA of ~7.3 kb length was obtained using reverse primer. PCR products of 3.87 kbps and 3.4 kbps respectively were amplified from cDNA. Nucleotide sequencing of the PCR products up to 450 base reaction was carried out using primers synthesized for the study.

II. Two fecal specimens collected from acute phase hepatitis A patients were inoculated one each in BGMK and Vero E6 cell lines. The inocula were HAV RNA positive by RT-PCR and for virus like particles by immune electron microscopy. Serial passaging of cell lines is being carried out for adaptation of HAV. BGMK cell line showed positivity to HAV by antigen capture ELISA at passage level 11(S/N ratio- 4.38). In Vero E6 cell line presence of HAV was detected at passage level 3 (S/N ratio 4.8).

## Future plan

HAV strains adapted to cell cultures will be characterized at genomic level. The strains will be purified and checked for immunogenicity in laboratory animals.

## Additional studies

### 1. Development of a Real time PCR assays for quantitation of viral nucleic acids of HBV and HCV

HBV DNA quantitation is used extensively for monitoring disease progression and efficacy of antiviral treatments during chronic HBV infection. Commercial assays for HBV DNA quantitation are available. However, considering relatively low sensitivity, limited dynamic range and cost of such assays, a real-time PCR assay was standardized using TaqMan minor groove binder probe and primers corresponding to HBV pre-core region. A 228bp fragment from this genomic region of HBV was PCR amplified, TA-cloned in pGEM-T-Easy vector (Promega) and serial dilutions of this DNA were used as a DNA standard. The assay showed wide dynamic linear range between 10-$10^9$ copies/ reaction with coefficient of regression 0.99 in all the experiments. Detection limit was as low as 10 genomic copies / reaction with low intra-assay (1.7%) and interassay (4%) variations. Comparison of the real-time PCR quantitation results from 35 clinical serum samples with those obtained by AMPLICOR HBV DNA monitor kit (Roche Diagnostics) revealed a significant correlation for all the samples (r=0.9). Though sensitivity, specificity and accuracy of real-time PCR and AMPLICOR assays were comparable, real-time PCR assay was found to have a broader dynamic range. At higher (>$10^7$) as well as at lower (<$10^3$) viral loads the results obtained by real-time PCR assay were more reproducible. Secondly, the samples with higher load needed to be diluted in the AMPLICOR assay for the accurate quantitation of HBV DNA, which increased the cost significantly. In conclusion, the real-time assay reported here provides an ideal alternative for HBV DNA quantification covering a large dynamic range of HBV DNA in a single, undiluted sample.

HCV RNA:

A 255 bp partial 5'NCR region of HCV genome was PCR amplified and TA-cloned. Clone with sense orientation was cut with SaI1 and run off transcription was done with T7 polymerase. Transcript was purified, serially diluted and used as RNA standard. Primers and probe corresponding to HCV 5'NCR were used and real time RT-PCR assay was performed. Serial dilutions of the standard RNA were used to see the linearity of the standard curve. Standard curve showed linear relationship from 9-$10^9$ RNA copies/ reaction. Sensitivity and reproducibility of the assay would be compared with a commercially available quantitation kit and then used to determine the viral load in clinical specimens.

### 2. Hepatitis C virus Interferon sensitivity determination region (ISDR) analysis

Several studies have demonstrated a correlation between outcome of antiviral interferon (IFN) therapy or IFN plus ribavarin combination therapy and the amino acid sequence of a small region, IFN sensitivity-determining region (ISDR, 237-276) of the HCV nonstructural protein 5A (NS5A). NS5A has been shown to interact with a cellular protein kinase, PKR, a primary mediator of the IFN-mediated antiviral response through the PKR-binding domain (40-amino-acid ISDR and 26 amino-acids downstream of the ISDR). Further downstream V3 (variable) region has also been documented in some studies as an important predictive region. Mutations in these regions have been associated with sensitivity to IFN. We studied pretreatment sera of 28 individuals (14 infected with genotype 3a, 4 with 3b, 5 with 1b, 5 with 1a). These patients are being monitored for the clearance of virus. The sequences of amino acid positions 2235-2425 in NS5A were determined. On the basis of preliminary studies, no correlation could be confirmed between the outcome of IFN therapy and the ISDR, PKR and V3 sequences in the individuals infected with HCV genotype 3b and 1b. However therapy responders, infected with genotype 1b, showed glutamine to arginine conversion at 289 position (PKR domain). Responders, infected with HCV genotype 3a showed four or more mutations in V3 region while nonresponders displayed less changes. Responders from genotype 1a group showed more than 5 changes in ISDR, PKR and V3 together while those in nonresponders were less than three. Since all the individuals are being followed further, final conclusions will be drawn only after monitoring the patients at least for 48 weeks.

### 3. Epitope mapping of HCV using random phage display library:

Epitope mapping of HCV is being done using random M13 phage display library. The main objective is to use these peptides along with HCV antigens in the development of diagnostic test for HCV. Subtractive biopanning was carried out by using pool of IgGs from normal and HCV infected individuals. Total 52 clones isolated from three different panning experiments resulted in identifying 7 different epitopes or antigenic determinants, three in core region (aa 74-79, 70-75, 231-235), two in NS5A region (aa 112-115, 316-324), one in E1/E2 region (aa 170-175) and one in E1 region (aa 122-126). These peptides would be synthesized and checked in ELISA using sera from HCV infected individuals with different anti-HCV antibody levels and with the sera from individuals infected with different genotypes of HCV. Peptides showing high reactivity will be included in the HCV diagnostic test.

### 4. ICMR funded clinical trial entitled, "Therapy in patients with chronic hepatitis C: A randomized control trial of interferon with ribavirin and combination of interferon with Glycyrrhizin."

For the above trial, NIV is one of the collaborating centers with responsibility of all the virological monitoring. During the last year, a total of 365 samples for 104 patients were tested for HCV RNA. Core region amplification was done for 51 patients. Sequencing for 42 samples has been completed. The genotypes were 1a (n=4), 1b (n=5), 1c (n=2), 3a (n=16), 3b (n=9), 3f (n=2), 3g (n=1), 3i (n=2). Quantitation of HCV RNA of 81 pre and post therapy samples was carried out using Amplicor HCV Monitor test (Roche diagnostics).

### 5. Analysis of membrane filters

Virological analysis was carried out for membranes prepared by a private enterprise using HAV as the model virus. The results were extremely encouraging as the filter was able to retain the virus at higher flow rate as well.

### 6. Assessment of DPTB vaccine from Serum Institute of India

The serum Institute of India has produced a quadruplet vaccine containing DPT and hepatitis B. Clinical trial of this vaccine was undertaken at two centers. At the NIV all the pre (n=604) and post-vaccination (n=604) were tested for antibody titres against all the four components of the vaccine in ELISA.

### 7. Hepatitis A with Guillain-Barré Syndrome A case study

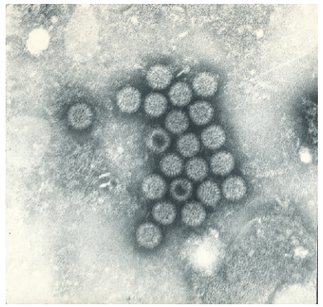
A case of acute of hepaptitis A with Guillain Barré Syndrome (GBS) in a 17-year-old male is reported. The patient presented with icterus flaccid quadripledgia and depleted motor response clinically. The electrodiagnostic examination classified patient as GBS subtype AMAN (Acute motor axonal neuropathy). The acute infection of viral hepatitis A resolved earlier than neurologic dysfunctions which lasted for >4 months. Cerebrospinal fluid was detected positive for anti-HAV,IgM, IgG, IgA, however, it was negative for HAV RNA by RT-PCR. The findings suggest the possible association of GBS with preceding HAV infection.

HAV RNA positivity was detected in serum and feces by RT-PCR indicating prolonged viremia and fecal excretion of HAV in the patient respectively. Phylogenetic analysis of sequences obtained for partial VP1/2A junction (168 bp) revealed HAV strain of genotype IIIA.





# Rotavirus



## 1. Epidemiology of Group A rotaviruses.

### Isolation and analyses of rotaviruses from children hospitalized for severe diarrhoea.

**SD Kelkar** nivrota@yahoo.com
PS Awachat, SS Ranshing

Fecal specimens were collected from hospitalized diarrhoea cases from Pune, India during the year 1990-1997, by NIV, Pune. Out of 432 rotavirus positive fecal specimens 47.92% could not be serotyped by monoclonal antibody (MAb) against G1-G4, G6, G8 and G10 serotypes, and were reported as nontypeable. In addition, about 10.88% fecal specimens showed multireactivity for more than one serotype. Seven nontypeable and four multireactive fecal specimens for rotavirus were inoculated in MA-104 cell line for virus isolation.

### Objectives

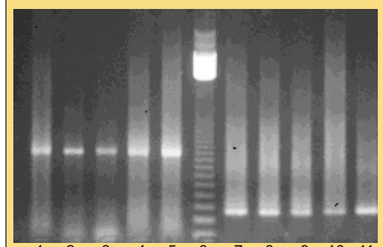
To characterize rotavirus isolates from Pune by molecular and serological methods.

### Achievements

#### Characterization of rotavirus isolates from nontypeable fecal specimens

Six rotavirus nontypeable specimens were successfully culture adapted and characterized as G9 strains by MAb ELISA (reported earlier). They were further confirmed as G9P[8] strain by RT-PCR. Four isolates were sequenced and were found to be 96%-99% homologous to AU32 strain of G9 reported earlier from Japan in 1985.

**Detection of culture adapted G9 isolates by RT-PCR**




**Lanes 1-5 (First PCR Products (1062 bp) of the Isolates 938048, 941659, 942835, 938452 & G9 F:45 prototype strain respectively).**
Lane 6 -123 bp Molecular Weight marker.
Lanes 7-11 (Nested PCR products (306 bp) of the Isolates 938048, 941659, 942835, 938452 & G9 F:45 prototype strain respectively).

#### Characterization of rotavirus isolates from multireactive fecal specimens

Rabbits were immunized with all four isolates and immune sera were obtained. The sera were tested for ELISA antibody levels. All sera showed ELISA titre of 1:80,000. Neutralizing antibody (NAb) levels against various rotavirus serotypes were determined.

#### Neutralization Assays

All four isolates were neutralized with antisera against BOV-LIN as well as NCDV strains belonging to G6 serotype at 10 fold higher titer than with antisera against other serotypes.





**Result of neutralization assays of G6 isolates**

| Tissue Culture Isolates | NAb titers with hyperimmune rabbit antisera to rotavirus serotype. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | G1 (KU) | G2 (S2) | G3 (YO) | G4 (ST-3) | G5 (OSU) | G6 (Bov.lin) | G6 (NCDV) | G8 (69 M) | G9 (F-45) | G10 (B223) |
| 9218021 | 50 | <10 | -ve | -ve | 50 | **1000** | **600** | 50 | 20 | <10 |
| 939303 | 100 | <10 | 10 | 20 | 10 | **5000** | **>5000** | 20 | 100 | ND |
| 939304 | 50 | 20 | -ve | <10 | 10 | **800** | **2000** | <10 | 50 | 10 |
| 9310295 | =10 | 50 | 50 | 50 | =100 | **2000** | **1000** | 20 | 20 | 50 |

[a] Neutralization titers are expressed as the reciprocal of the highest dilution of rabbit antiserum that showed more than 50% neutralization with 100 ELISA End Point (EEP) of virus/ isolate used in the 'N' Ab assay.
-ve, Neutralization was not obtained even with undiluted serum samples

The results of neutralization assay of reference rotavirus serotypes against rabbit hyperimmune antisera to the four isolates are presented in the following table. All four isolates developed high titere NAb to the G6 serotype. The antisera raised against all isolates neutralized serotype Bov. Lin. at very high titers than all other rotavirus serotypes. The cross neutralization assays confirmed the identity of isolates as G6.

**Results of neutralization assays using hyper immune antisera raised against G6 isolates**

| Antisera to tissue culture isolates | **NAb titers[a] with immune sera against tissue culture isolates to rotavirus G serotypes** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 (KU) | G2 (S2) | G3 (YO) | G4 (ST-3) | G6 (Bov.Lin.) | G8 (69M) | G9 (F-45) | G10 (ST-10) |
| 939304 | < 50 | 200 | 50 | 100 | 6000 | >400 | < 50 | < 50 |
| 9310295 | < 50 | 200 | < 50 | 100 | 8000 | 100 | < 50 | < 50 |
| 939303 | < 50 | 200 | < 50 | 100 | 8000 | < 50 | 50 | < 50 |
| 9218021 | < 50 | 100 | < 50 | 200 | 8000 | < 50 | 50 | < 50 |

[a] Titers are expressed as described below Table I.

### Future plan

G6 isolates will be further characterized by molecular methods.

Rota virus transmission by faeco oral route

## 2. Investigation of gastroenteritis outbreaks

**SD Kelkar** nivrota@yahoo.com
**PS Awachat, JK Zade**

A widespread diarrhoea outbreak occurred during the year Dec 2000-Jan 2001 at Jawhar, Thane district Maharashtra, India. Four hundred ninety patients were hospitalised. Twenty-seven out of 39 (69.23%) fecal specimens were detected positive for rotavirus by ELISA. Of these, four fecal specimens were undertaken for isolation and characterization of rotaviruses.
In an outbreak of diarrhoea occurred in 2000 at Daman, Union Territory of India, situated on the west coast of India, a total of 352 cases were reported. Sixty nine cases were hospitalized. Although majority of the cases occurred among adults, children were also affected. The disease was less severe as compared to adult diarrhoea epidemics in China. There was no mortality.

### Objectives

- To isolate and characterize rotaviruses involved in diarrhoea outbreak in tribal population at Jawhar
- To determine the immune status against rotaviruses in tribal population of Jawhar
- To detect and characterize a causative agent of diarrhoea outbreak at Daman

### Achievements

**Isolation and characterization of rotaviruses causing diarrhoea outbreak**

Four group A rotavirus positive fecal specimens, belonging to children aged 3 months to 2 years, suffering from diarrhoea at Jawhar in the year 2000 were taken for virus cultivation. Three isolates were detected between passages 4 and 8. MAb ELISA serotyped the isolates as G3. Hemagglutination tests carried on two isolates (006956 & 006964) showed agglutination of guinea pig erythrocytes at 1:16 dilution indicating presence of rotavirus of animal origin. Two isolates were confirmed as G3 serotype, by combined tissue culture and ELISA based neutralization test.

**Characteristics of rotavirus G3 strains isolated from fecal specimens from an outbreak at Jawhar.**

| | Fecal Specimens | | | | Tissue culture isolates | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fecal Sp. No. | Sub Group | RNA pattern | PCR | Isolate No. | Sub Group | RNA pattern | MAb ELISA | PCR | HA |
| FS-006956 | II | Long | G3 | I-006956 | I&II | Long | G3 | G3P[8] | 1:8 |
| FS-006959 | ND | Long | G3 | I-006959 | I&II | Long | G3 | G3P[8] | ND |
| FS-006964 | Non I NonII | Long | G3 | I-006964 | II | Long | G3 | G3P[8] | 1:16 |

HA: Hemagglutination; ND: Not Done

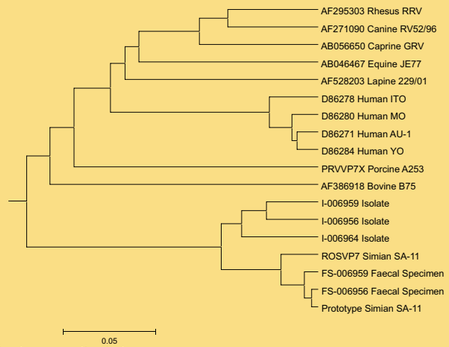
One isolate 006964 neutralized hyper immune serum against human G3 serotype at >1:5000 dilution and the G3, Simian (SA-11) virus at 1:20000 dilution. The other isolate 006956 neutralized human G3 serum at 1:1000 dilution and G3 and simian (SA11) serum at >1:10000 dilution. All three isolates were confirmed as G3 type by RT-PCR. The nucleotide sequencing





of the 582 bp nested PCR products of the culture adapted isolates and two of their original fecal specimens showed 96% to 98% homology with Simian G3, SA11 rotavirus, thereby confirming a G3, simian SA11 like rotavirus as etiological agent of the diarrhoea outbreak.

A phylogenetic tree showing relationship between tissue culture isolates, fecal specimens from Jawhar, simian G3, SA11 strain, and various human, animal G3 rotaviruses.




### Serological studies in the tribal population

In order to detect neutralizing antibodies (NAb) against G3 (SA11) serotype, following serum samples were collected in Jawhar: Acute serum samples from 15 children, convalescent serum samples from 7 child patients and their mothers (n=10) 2 years after an outbreak, fifty sera from healthy adult females who visited hospital for anti-natal and post-natal care and sera from 7 healthy children.

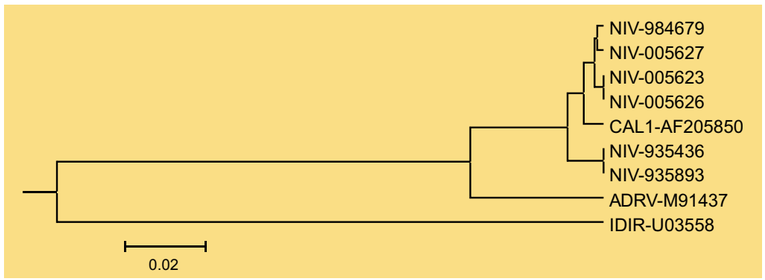
Fifteen acute serum samples from child patients showed geometric mean titre (GMT) of NAb 1:10 to simian G3 (SA11) serotype. Seven convalescent serum samples of the child patients showed GMT of NAb 1:70 against G3 serotype. Six of 7 child patients showed four fold high NAb titres against G3 simian SA11 in convalescent sera. Mothers of 10 pediatric cases showed GMT of NAb 1:80 against G3 SA-11. Forty sera collected from healthy adult females showed GMT of NAb 1:22.

Thus, adult female population showed low level of NAb as compared to mothers of children who suffered from diarrhoea indicating subclinical infection. The above results indicate that low NAb against G3 serotype among the tribal population may have led to an outbreak due to G3 simian like serotype.

### Characterization of an etiological agent of diarrhoea outbreak occurred in Daman

Five faecal specimens from hospitalized diarrhoea patients from Daman, were received at NIV, Pune. All were tested negative for group A rotavirus by ELISA. Group B rotaviruses were detected in three out of five faecal specimens by RNA PAGE and RT-PCR. Nucleotide sequencing of the nested PCR products confirmed that all three group B strains detected were close to NIV 984679 and CAL-1 strains reported earlier from Pune and Kolkata in 1998.

Phylogenic relatedness of rotavirus isolates from present study




## Future plan

The G3 isolates will be further characterized by P typing..

Molecular epidemiological study of group B rotavirus will be undertaken.





## 3. Sero-epidemiology of rotavirus infection

**SD Kelkar** nivrota@yahoo.com
**PG Ray**

IgM antibody that appears first following rotavirus illness is considered as a marker of primary infection, while serum IgA/IgG levels have been correlated with resistence to severe rotavirus illness.

Data on rotavirus specific immunoglobulins among mothers at delivery and its relation to development of rotavirus infections and diarrhoea among infants are lacking in India. Serological studies on postpartum serum and milk samples may provide useful information to assess the extent of exposure to rotavirus and subsequent development/maintenance of anti-rota virus antibody in different socio-economic groups of mothers.

## Objectives

- To estimate anti rota IgM/IgG/IgA antibody levels in children with rotavirus/non-rotavirus diarrhoea and their mothers
- To detect rotavirus specific IgM antibody levels in mothers' and infants from higher socio-economic (HSG) and lower socio-economic (LSG) groups.

## Achievements

### Comparative studies on serum antirotavirus antibodies among children following rotavirus diarrhoea/non-rotavirus diarrhoea and their mothers

Results on rotavirus specific IgM & IgA levels have been presented in annual report 2002-03. Results on rotavirus specific IgG were as follows: In RVD group, 22/24 (91.66%) children and 9/24 (37.5%) mothers showed IgG seroconversion. In NRVD group 4/10 (40%) children and none of the mothers showed IgG seroconversion. Range of IgG titres in the both groups is shown in the table.

| | | Acute | Convalescent |
|---|---|---|---|
| RVD | Children | $5x10^3$ $25x10^3$ | $50x10^3$- $1x10^5$ |
| | Mothers | $16x10^3$-$20x10^5$ | $1.2x10^5$ - $25.6x10^5$ |
| NRVD | Children | $50x10^3$ $1x10^5$ | $50x10^3$ $1x10^5$ |
| | Mothers | $6.4x10^5$ $25.6$ $x10^5$ | $6.4x10^5$ $25.6$ $x10^5$ |

Acute and convalescent phase sera in RVD group showed significant difference in IgG titres as compared to NRVD group.

Project completed.

### Prevalence of rotavirus specific IgM in mothers and infants

Specimens were obtained from mothers admitted to the hospitals for normal delivery or planned caeserians. They included primies or mothers with one child. Samples from HSG and LSG were collected from Patankar nursing home and Bharati hospital respectively. The respective authorities were contacted and a written consent was obtained for the collection of

samples. The mothers enrolled in the study were interviewed individually and a detailed oral as well as written information regarding the study was provided to them. A case history form regarding details of each mother infant pair was duly filled soon after delivery. The following samples were collected for the proposed study:

- Cord blood sample after delivery (n=56 from each group)
- Mothers blood sample after delivery (n=56 from each group)
- Colostrum sample on 1st-3rd day of delivery (n=56 from each group)
- Milk sample on 4th-6th day/3rd month/6th month of delivery (n=56 from each group)
- Serum samples from mother-infant pairs at 6 months (n=17 pairs from each group).

Testing of rotavirus specific IgM was done by capture ELISA. Surveillance was maintained on all infants and their mothers by weekly telephone calls to know the feeding regimen and episodes of diarrhoea among their infants. Some of the mothers from the LSG could not be regularly followed-up due to inappropriate residential addresses and lack of communication. As expected cord blood samples were negative for rotavirus specific IgM antibodies. IgM positivity in mothers was in the range of 64%-75%. Statistically no difference was noted in the prevalence and geometric mean titres (GMTs) of anti rotavirus IgM antibodies in the mothers from two groups (P>0.05). IgM positivity in milk samples at 6 months was 52.94% and 82.35% from HSG and LSG respectively (P>0.05). IgM positivity in infants was significantly higher in LSG than HSG (94.11% vs 52.94%), (P <0.05).

**IgM positivity in mothers and infants from Pune, India**

| Specimens | HSG (Site 1l) | | LSG (Site 2) | |
|---|---|---|---|---|
| | No. positive/ No. tested (%) | IgM GMT | No. positive/ No. tested (%) | IgM GMT |
| Mothers serum (after delivery) | 42/56 (75.0%) | 250.92 | 36/56 (64.28%) | 466.61 |
| Mothers serum (at 6 months) | 13/17 (76.47%) | 163.11 | 11/17 (64.7%) | 288.66 |
| Mothers milk (at 6 months) | 9/17 (52.94%) | 63.85 | 14/17 (82.35%) | 90.62 |
| child serum (at 6 months) | 9/17 (52.94%) | 122.61 | 16/17 (94.11%)* | 339.8 |

* P<0.05

Infants were followed-up for rotavirus diarrhoea upto 6 months of age. The number of children who suffered diarrhoeal episodes from the LSG was significantly higher than that of HSG, (P <0.05) as shown in the following table.





**Follow-up for diarrhoea in children (n=17)**

| Groups | Absence of diarrhoea | Presence of diarrhoea |
|---|---|---|
| HSG | 11 (64.7%)* | 6 (35.29%) |
| LSG | 2 (11.76%) | 15 (88.24%)* |

* P<0.05

## Future plan

Rotavirus specific IgG and IgA levels will be estimated in cord/maternal blood, colostrum /milk samples at delivery and in follow-up milk/serum samples from mothers as well as sera from infants. Rotavirus will be detected in the follow up stool samples of children by ELISA/RT-PCR.

## 4.Characterization of rotaviruses

**SD Chitambar** nivrota@yahoo.com
**JK Zade, VS Tatte, SS Ranshing**

### Group A rotaviruses

Group A rotavirus has 14 serotypes based on VP7 antigen present on the outer capsid. Out of these, at least 10 are known to cause diarrhoea in children. Although MAbs for some of the serotypes are available, if sufficient double shelled particles are not present in the test specimens, the rotavirus serotype cannot be determined. RT-PCR is more sensitive method for serotyping as compared to MAb based ELISA.

### Objectives

- To detect group A rotaviruses by RT-PCR.
- To type and characterize group A rotaviruses and unusual/nontypeable rotavirus strains from fecal specimens of diarrhoea patients.

### Achievements

In order to detect group A rotaviruses by PCR, primers from VP6 gene which is conserved in all the group A rotaviruses was selected. Fecal specimens were collected from hospitalized children admitted at Bharati hospital, Pune. The specimens were grouped as rotavirus diarrhoea (RVD) and non-rotavirus diarrhoea (NRVD) on the basis of ELISA results. In order to examine the specimens belonging to NRVD group, RT-PCR was carried out. All the 13 specimens from NRVD group were tested by RT-PCR. It was found that 3 specimens, earlier grouped as NRVD were positive for rotavirus.

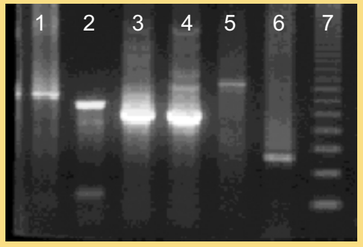
### Typying and characterization of group A rotaviruses

Serotyping of rotaviruses using monoclonal antibodies can be carried out by two procedures. In method A ELISA plates are coated with monoclonal antibodies and antigen is detected by polyclonal antibodies while in method B ELISA plates are coated with polyclonal antibodies and antigen is detected by monoclonal antibodies. Normally, method A is used for serotyping. It was noticed earlier that some fecal specimens which could not be serotyped by method A were positive for G2 serotype by method B. Such specimens (911838, 9218037, 9218621, 932588, 932631 and 933140) were tested by nested RT-PCR. The product of nested PCR from five specimens was sequenced and all specimens were confirmed as G2 serotypes.

Simultaneously, optimization of the conditions of multiplex PCR for group A rotavirus serotypes was initiated using primers specific to gene 9 encoding VP7 glycoprotein and tissue culture derived reference serotypes G1-G4,G8,G9 of human origin and G5,G6,G10,SA-11(G3) of animal origin as shown in the following figure.








Lane 1. HRV 1 (G1) 749 bp.
Lane 2. HRV-2 (G2) 657 bp.
Lane 3. SA-11 (G3) 582 bp.
Lane 4. Bovine Lincon (G6) 582 bp.
Lane 5HRV 8 (G8) 885 bp.
Lane 6. HRV 9 (G9) 306 bp.
**Lane 7 123 Bp Ladder**

### Future plan

Characterization of nontypeable/multireactive fecal specimens will be continued further.

## 5. Diagnostics for group A and Group B rotaviruses

**SD Chitambar** nivrota@yahoo.com
**SS Ranshing, VS Tatte**

### Introduction

The rapid ELISA for detection of group A rotavirus has been developed at NIV, Pune. Attempts at development of ELISA for group B rotaviruses are being made.

### Objectives

To develop ELISA for the detection of group B rotavirus.

### Achievements

An Indian patient entitled, “A process for preparing rotavirus immune serum conjugated with horse radish peroxidase (HRP) for use in rapid enzyme linked immunosorbant ELISA kit for diagnosis of rotavirus” has been obtained in Feb. 2004. Technology transfer to industry is in process.

ELISA to detect group B rotavirus was standardized using hyper immune sera raised against semipurified group B rotavirus recovered from specimen 005623 and anti-group B rotavirus IgG conjugated to HRP. One hundred non group A fecal samples collected in the year 1992 were screened by ELISA. Three samples showed P/N ratio more than 2. To confirm the presence of group B rotavirus, RNA-PAGE and RT-PCR were carried out. One of the three samples showed positivity in both the assays.

### Future plan

Non group A rotavirus fecal specimens collected from adults are cryopreserved for further studies.





## 6. Preparation of egg yolk antibodies against rotaviruses for passive immunization of humans and poultry

**Detection of avian rotavirus antibodies in birds**

**SD Chitambar** nivrota@yahoo.com
**B Manika, GS Dhale**
**Collaborator: G R Ghalsasi**

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

### Objectives

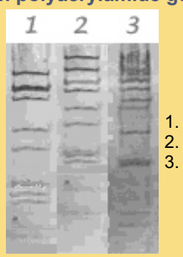
To develop an ELISA specific for detection of avian rotavirus antibodies.

### Achievements

Normal chicken sera were collected from the birds kept under captivity in NIV animal house and in PDRC, Pune. Hyperimmune serum raised against avian rotavirus strain CH2 was procured from PDRC. Using semipurified preparation of tissue culture grown CH2, indirect ELISA test was attempted to differentiate normal chicken and hyperimmune chicken sera. However, lack of specificity in the assay was observed. Therefore development of assays specific for detection of avian rotavirus and its antibodies was undertaken.

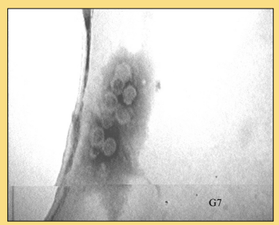
Reference avian strain (CH2) was grown on large scale in MA104 cell line. Cultures were harvested after observing cytopathic effect. The virus was characterized by RNA PAGE, EM and PCR (Figs.). After clarification by centrifugation, virus was pelleted by ultracentrifugation and purified through 20-60% continuous sucrose gradient. Fractions were collected, tested by ELISA for the presence of virus and pooled according to OD values and cryopreserved for further studies.

**RNA migration pattern on polyacrylamide gel**

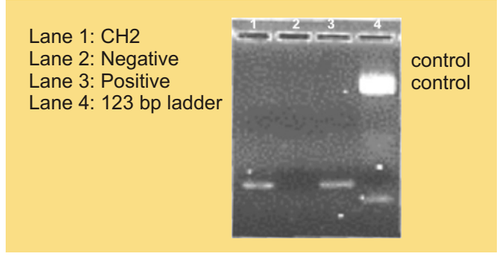



1. Human Rotavirus
2. Animal Rotavirus
3. Avian Rotavirus (CH2)

**Double shelled particles of CH2 rotavirus visualized under EM**



Electrophoretic migration pattern of PCR products on agarose gel (259 bp)




## Future plan

ELISA reagents such as hyper immune serum against CH2 and specific anti-CH2 immunoconjugate will be prepared. These will be subsequently used in various ELISA protocols for detection of avian rotavirus antibodies and virus/antigen.

## 7. Development of monoclonal antibodies against different rotavirus serotypes

**SD Kelkar** nivrota@yahoo.com
**GS Dhale**

In order to develop monoclonal antibodies against G9 rotavirus serotype, fusion was carried out between SP2 cells and spleen cells from G9 immunized mouse. Generation of one clone (P4E1-F6) has been reported earlier. Due to its non-specific reactivity with rotaviruses other than serotype G9, it was decided to reclone. Recloning generated two clones (B2, F6) which were tested by ELISA for neutralization against G9 and G3 (SA11) rotaviruses. Both the clones showed reactivity to G9 virus but not to SA-11 as shown in Table.

| Reclones of P4E1-F6 | OD in antibody detection ELISA | |
|---|---|---|
| | G9 | SA-11 |
| B2(W1) | 0.687 | 0.039 |
| B2(W3) | 0.786 | 0.041 |
| F6(W1) | 1.620 | 0.044 |

However, further expansion of the clones again showed non-specific reactivity and therefore these clones were cryopreserved.





## 8. Measurement of anti-rotavirus antibodies in hyper immune goat sera and colostrums

**SD Kelkar** nivrota@yahoo.com
**VS Tatte, SS Ranshing**

As an approach to confer the protective immunity against rotaviruses passively, hyper immune goat colostrums were prepared against human rotavirus serotypes G1-G4, G8 and G9 with booster dose of animal rotaviruses belonging to different serotypes viz. G3 (Simian virus), G5 (Porcine), G6 & G10 (Bovine). Transplacental transfer of antibodies does not occur in goats. Serum levels of antibodies in kids are indicative of antibody titres attained after suckling on to mother goats. Therefore, for estimation of anti-rota antibodies in goat kids, sera were utilized.

## Objectives

To assess anti-rotavirus antibodies in hyper immune goat sera and colostrums

## Achievements

Serum anti-rota IgG antibodies:

IgG antibodies to rotavirus in hyper immune sera were estimated by ELISA. Mother goat sera showed GMT values of 1:688 and 1:27825 for pre and post sera respectively. Goat kids suckled on to these mothers showed GMT value of 1:2824.

Colostrum and serum anti-rota neutralizing antibodies:

Sera from 12 goat kids were collected one month after suckling on to mother goats. Respective mother goats were also bled and sera collected. The pre sera 'N'Ab titres in mother goats had GMT value of 1:100 and the post sera 'N'Ab titres had GMT value 1:4000. GMT of kids 'N'Ab titre was 1:2000.

The colostrum pools prepared against various rotavirus serotypes were tested for homologous and heterologous neutralizing antibody titers. The results are shown in the table.

NAb titers of colostrum pools against human rotavirus serotypes

| Schedule/ No. of Goats | Immunizing virus (human/animal | 'N'Ab titers against prototype strains | | | | |
|---|---|---|---|---|---|---|
| | | G1(KU) | G2(S2) | G4(ST-3) | G8(69M) | G9(F45) |
| S/3 | G8(69M) boosting with G10 (B223) | 1000 | 1000 | 1000 | 7000 | 1000 |
| T/3 | G9(F45) boosting with G5(OSU) | 5000 | 3000 | 5000 | 5000 | =10,000 |
| U/4 | G4(ST-3) boosting with G6 (BOV-LIN) | 8000 | 1000 | 6000 | 10,000 | =10,000 |
| V/1 | G1,G2 (913700) boosting with G3 (SA-11) | 8000 | 8000 | 5000 | 8000 | 7000 |

From the above results the NIV isolate, 913700 which is dually reactive to G1 & G2 MAbs seems to be promising.

From the above results the NIV isolate, 913700 which is dually reactive to G1 & G2 MAbs seems to be promising.

**Colostorum anti-rota IgA antibodies**

Colostrum pools were tested by IgA class capture ELISA for anti-rota IgA antibody. The test was standardized by using anti goat IgA raised in rabbit as capture antibody. All pools tested showed approx titres of 1:3200.





# Influenza

Virus isolation in tissue culture

## 1. Epidemiology and investigation of influenza outbreaks in Pune city

**BL Rao** flunetpune@yahoo.com
**LR Yeolekar, SS Kadam, MS Pawar, PB Kulkarni**

National influenza Center at NIV, Pune is the main center in the country conducting continuous surveillance on influenza during the past 27 years.

### Objectives

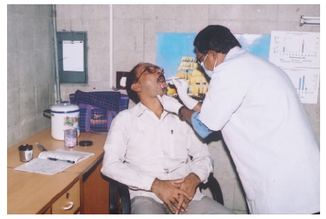
- Surveillance for influenza
- Virus isolation and strain characterization.

### Achievements

During the course of this surveillance in the year 2003, specimens were collected from two hospitals and five dispensaries located in different areas of Pune. A total of 573 throat swab/nasal swab (TS/NS) and 190 nasopharyngeal aspirates (NPA) were collected from patients attending outpatient departments and from patients admitted to inpatient ward. Majority of the cases were children. 507 out of 573 TS/NS specimens and 42 out of 190 NPA specimens were processed in MDCK cell culture and 37 influenza virus isolates; 23 A(H3N2) and 14 type B were isolated. The type A(H3N2) isolates were identified as related to A/Panama/2007/99 (H3N2) strain and type B isolate as related to A/Beijing/184/93 (B/yamagata lineage) strain in HI test.

Strains of influenza type B belonging to B/victoria lineage were isolated at NIV, Pune in 2002 whereas all the type B strains isolated in 2003 belonged to the B/yamagata lineage. Three type A (H3N2) isolates and one type B isolate were sent to WHO Influenza Collaborative Centre, CDC, Atlanta, USA. They were characterized as similar to A/Panama/2007/99 like (Jan., March H3N2 isolates), A/Korea/770/2002 like (August H3N2 isolate) and B/Sichuan/379/99 like strains.

Increase in influenza like cases was noted in 2 peaks, March-April (summer season) and July-August (rainy season) with maximum number of isolates obtained during these peaks. These seasonal peaks of influenza activity have been commonly observed during the past 25 years of influenza surveillance in Pune. Influenza virus isolates were obtained from all the health centers included in the study, which indicated influenza strains were circulating in different localities of Pune city.



Throat swab collection

**Monthly data of specimen collection and influenza virus detection during 2003**

### Future plan

NIV being National Influenza Centre, year round surveillance for influenza will be continued.





## 2. Rapid detection and strain analyses of influenza viruses using molecular techniques.

**LR Yeolekar** flunetpune@yahoo.com
**SD Pawar, BL Rao**

For rapid identification of new variant strains emerging in the community and to expand and upgrade the existing surveillance system, molecular and rapid diagnostic techniques for detection of newly emerging strains are being developed.

### Objectives

- Development of molecular and rapid diagnostic techniques such as ELISA, RT-PCR and their application for diagnosis of influenza cases.
- Development of monoclonal antibodies for rapid detection using antigen capture ELISA.
- Standardization of molecular methodologies for genetic analyses of virus isolates and rapid identification of variant strains.

### Achievements

#### a. Antigen capture ELISA for detection of influenza A and B viruses.

As reported previously influenza viral antigens from infected tissue culture fluid were detected with 100% sensitivity and specificity when compared with HI test. However, 43.7% sensitivity was observed for detection of influenza viral antigen from clinical specimens as compared to virus isolation. Attempts to improve the sensitivity and specificity were made using following modifications a) Addition of Triton X-100 to a final concentration of 0.1% to the clinical specimens. b) Sonication of Triton X 100 treated samples with pulse of 9.9 seconds with a gap of 2 seconds for 5 min in a 1.5 ml microfuge tube floated in a cup horn assembly containing ice water. c) Substrate OPD, UP was replaced by TMB, $H_2O_2$ and read at 450 nm.

#### Influenza A virus

Of the 486 samples subjected to virus isolation and ELISA for influenza A virus, 23 samples yielded virus isolates and 24 samples were positive in ELISA. The sensitivity and specificity is indicated in the table.

**Detection of influenza A antigen from clinical samples: Comparison of ELISA with virus isolation**

| ELISA | Influenza virus isolation | | |
|---|---|---|---|
| | + | - | |
| + | 19 | 5 | 24 |
| - | 4 | 462 | 466 |
| | 23 | 467 | 490 |

Sensitivity: 19/23= 82.6%
Positive predictive value: 19/24=79.1%
Specificity: 462/467=98.9%
Negative predictive value: 462/466=99.1%

This indicates that lysis/de-aggregation of the virus particles using Triton X 100 and sonication improved the sensitivity of the test when compared with virus isolation.

Indigenously developed ELISA was used for detection of influenza A virus in 30 TS/NS samples collected during an outbreak at Murshidabad, and compared with RT-PCR. Of the 30

samples tested for influenza A virus, 15 were positive in ELISA. Comparison of ELISA and RT-PCR is shown in the table.

**Detection of influenza A antigen from Murshidabad samples: Comparison of ELISA with RT-PCR.**

| ELISA | RT-PCR | | |
|---|---|---|---|
| | + | - | |
| + | 11 | 5 | 15 |
| - | 5 | 10 | 15 |
| | 16 | 14 | 30 |

Sensitivity: 11/16= 68%
Positive predictive value: 11/15=73%
Specificity: 10/14=71%
Negative predictive value: 10/15=66.6%

Samples were also subjected to virus isolation. All the four isolates obtained were from samples that were positive in ELISA of which, three were positive and one negative in RT-PCR. Considering the small number of samples used for this comparison, further work on larger number of samples is in progress.

## Influenza B virus

Of the 490 samples subjected to virus isolation and ELISA for influenza B, no correlation between ELISA positivity and virus isolation was observed. Also, of the 30 samples collected during an outbreak in Murshidabad, when screened for influenza B viral antigen, 10 samples were found positive. However, all ten samples were negative for virus isolation as well as in RT-PCR for influenza B. This indicated that the test was giving nonspecific results and couldnot be used for the detection of influenza B in its present form.

## b. RT-PCR for detection of influenza A, B and Respiratory Syncytial Virus (RSV)

### Detection from tissue culture fluid

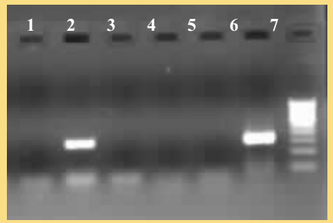
Development of RT-PCR for detection of influenza A, B and RSV was undertaken. Specific primers to the matrix (M) gene for influenza A, non structural (NS) gene for influenza B and fusion (F) gene for RSV were synthesized to give an amplicon of 311 bp, 226 bp and 380 bp respectively. A semi-nested PCR giving an amplicon of 247 bp, 207 bp and 319 bp for influenza A, B and RSV respectively was also standardized. Infected MDCK stocks or egg allantoic fluid of influenza A and B viruses and B-SC-1 or HEp-2 stocks of RSV were used. Normal cell lysates of respective cell line or normal allantoic fluid were used as controls.

Influenza type A(H1N1) (NIV No. 003150), A(H3N2) (NIV No.032261), type B (NIV No. 021396 and 945141) and RSV (Reference A2 strain, NIV No.955879) could be detected by RT-PCR. Semi-nested PCR increased the sensitivity of detection 10-100 fold for all three viruses.

### Detection from Clinical samples

Twenty-seven clinical samples (22 NPA, 5 TS) collected during 2002 which included two samples positive for influenza B virus isolation, one each positive for influenza A and RSV isolations were screened for detection of influenza A, B and RSV by RT-PCR. A semi-nested PCR was performed. Of the 27 samples screened, the specimens positive for virus isolation were also positive in RT-PCR. In addition one each negative for influenza A and RSV were positive by RT-PCR. Semi-nested PCR products (319 bp) of two specimens positive for RSV were sequenced. Sequence of both products showed 99% and 97% homology with Human RSV isolate LLC62-111 confirming specificity of the test.

**RT-PCR on clinical samples for the detection of influenza A virus**




311 bp

Lane
1,2,3,4,5: Clinical specimens
6: Positive control
7: Marker (100bp)

## c. Genetic analysis of virus isolates

RT-PCR for the identification of subtypes of haemagglutinin (H1 and H3) and neuraminidase (N1 and N2) genes of influenza A virus has been standardized. Since 1977 A(H3N2) and A(H1N1) sub-types of influenza A virus have been co-circulating in the community. Emergence of A(H1N2) subtype of influenza A due to re-assortment of the co-circulating strains was reported the World over in 2000 . Three influenza A strains isolated in the year 2000 at NIV (NIV#003150, 003827, 003562) identified as having H1 sub-type by HI test were identified as having N1 subtype by RT-PCR.

## d. RT-PCR for detection of H5 subtype of influenza A virus

An outbreak of avian influenza A(H5N1) occurred in South-east Asia in Jan 2004. The highly pathogenic virus spread to poultry in 10 countries. It crossed the species barrier in Thailand and Vietnam causing disease in 32 persons, of which 22 died. There was no report of H5N1 outbreak in poultry from India.

RT-PCR for detection of H5 subtype of influenza was standardized at NIV. Primers specific to the HA gene of H5 subtype recommended by WHO giving on amplicon of 380 bp and nested sets designed at NIV were synthesized. BPL inactivated strains of H1, H3 and H5 subtypes and type B influenza virus antigens supplied by WHO were used for standardization.

An amplicon of 380 bp was obtained with H5 antigen. Non-specific reactivity with H1, H3, type B and normal antigen was not observed. Sequencing of the 380 bp amplicon was conducted and showed 99% homology with HA gene of A/Duck/Postdam/1402-6(H5) influenza virus. All nested primers reacted with the first PCR product given amplicons of estimated size. This test could easily be used for rapid detection of influenza H5 subtype.

## e. Development of monoclonal antibody for Influenza

NIV has undertaken the development of indigenous monoclonal antibody based rapid diagnostic tests for influenza diagnosis. Preparation of MAbs against an Indian influenza virus isolate, 8912370 A (H3N2), similar to A/Sichuan/68/89 was undertaken.

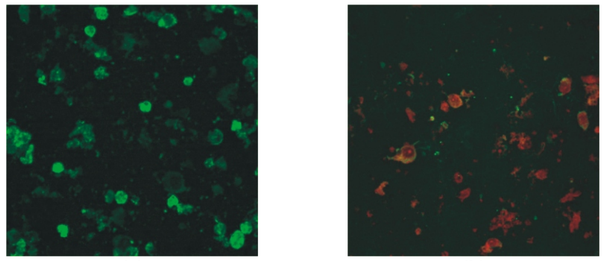
Characterization of MAbs from three hybrids viz. 3F1, 4F11, and 6A11 was undertaken. All immune peritoneal fluids (IPFs), tissue culture fluids (TCFs) were screened to test the presence of MAbs. All the IPF as well as TCF aliquots showed presence of MAbs.

These MAbs were titrated in ELISA and MAb titres in IPFs and TCFs were in the range of $10^6$-$10^8$. Isotyping of MAbs showed that 3F1 and 4F11 are of IgG1 subtype and MAb from 6A11 clones was of IgG2a subtype.

MAbs were tested in hemagglutination Inhibition (HI) test with A/PR/8/34 A (H0N1), A/New calidonia/20/99 A (H1N1), A/Singapore/1/57 A (H2N2), A/sichuan/68/89 A (H3N2)-homologous virus against which MAbs were raised, A/Sydney/5/97 A (H3N2)-WHO supplied antigen, A/Sydney/5/97 A (H3N2)-NIV isolate, B/Harbin/7/94, and B/Beijing/184/93 like Type B influenza viruses. None of the tested MAb showed HI activity indicating that these MAbs do not interfere with the HA activity of influenza viruses.

Western blot analysis of these MAbs showed reactivity of these MAbs with influenza virus nucleoprotein (NP). IPFS from two clones from each hybrid were tested in immunofluoresence (IF) test with homologous A(H3N2) 8912370 virus (following figures). All the MAbs showed positive reaction in IF using confocal microscope.

**Immunofluorscence using NIV-Mab on (a) infected and (b) normal MDCK cells**



(a) (b)

Antigen capture ELISA (AC-ELISA) was standardized using indigenously developed Flu-A MAbs (NIV-MAb). These MAbs were used for capturing and antibody used for detection was rabbit polyclonal antibody raised against influenza A(H3N2), which is similar to A/Sichuan/68/89. Reactivity of ten influenza type A subtypes, different strains of type B influenza virus and other respiratory viruses viz. respiratory syncytial virus (RSV), parainfluenza-1,2,3 and adenovirus were tested in AC-ELISA. The subtypes included human, equine and swine influeza type A strains and were A(H1N1), A(H2N2), different strains of A(H3N2), A(H4N2), A(H5), A(H6), A(H9N2), A(H7N7), A(H3N8), A/Swine/30. The AC-ELISA could detect all the ten subtypes of influenza type A where as influenza type B, and other respiratory viruses were not detected. Thus the test was type specific for the detection of type A influenza virsues.

## Future plan

Molecular techniques will be used to screen clinical samples and characterization of influenza isolates.





## 3. Monoclonal antibody (Mab) based IF test and ELISA for detection of Respiratory Syncytial virus (RSV) infection.

**RG Damle** flunetpune@yahoo.com
**LR Yeolekar, MR Khude, BL Rao**

RSV is the single most important cause of hospitalization for serious respiratory tract viral disease in infants and young children Worldwide. It is also suspected as a possible cause of childhood asthma. RSV has been deemed most important for vaccine development.

NIV has developed MAbs against 955879 strain of RSV isolated at NIV. Rapid diagnostic tests viz. ELISA and immunofluorescence (IF) test employing these indigenous MAbs have been developed and evaluated for the detection of RSV infection from clinical samples.

## Objectives

Epidemiological investigation of RSV in Pune employing MAb based immunodiagnostic tests developed at NIV.

## Achievements

### a) Detection using indigenous MAbs

During 2003, 169 nasopharyngeal aspirates (NPA) were collected, from cases with acute respiratory infection, 99 attending outpatient department (OPD) and 70 from those admitted to inpatient department (IPD) of KEM Hospital, Pune. Cells from NPA were subjected to immunofluorescence (IF) test for detection of RSV using indigenously developed MAbs against RSV. 2 samples collected in December 2003 showed presence of RSV antigen. Interestingly, no RSV activity was observed in the months of August September as was observed in the previous year even though the number of cases visiting KEM Hospital suffering from acute respiratory disease showed similar pattern in both years.

### b) Detection using commercial kit

Samples collected from KEM hospital were also subjected to IF test using Commercial Kit (Chemicon) for the detection of Influenza A, B, RSV, Parainfluenza 1,2,3 and Adenoviruses and some were also inoculated in MDCK or HEp-2 cells for virus isolation.

Of the 131 samples screened, 8 samples showed presence of virus/viral antigen for influenza A, four for influenza B, two for RSV, one for parainfluenza-1, two for parainfluenza-3 and one for adenovirus. Five samples were positive for respiratory virus panel pool but did not have sufficient cells for further identification. Eleven influenza isolates and one adenovirus isolate were obtained.

Immunofluorescence test for detection of respiratory viruses

**Detection of different respiratory viruses**

## Future plan

Epidemiological investigation of RSV infection in Pune will be continued employing MAb based immunodiagnosis tests developed at NIV

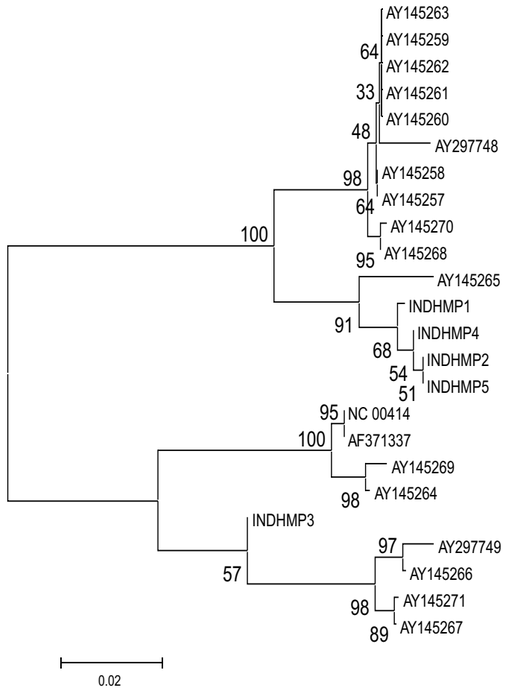
## 4. Molecular detection of human metapneumo virus (HMPV) infection in children with acute respiratory infection in Pune, India.

**B.L. Rao.** flunetpune@yahoo.com
**SS Gandhe, SD Pawar, VA Arankalle**

In 2001, Van den Hoogen *et al* first reported the discovery of Human Metapneumovirus (CHMPV) in respiratory patients from the Netherlands. The disease spectrum ranged from mild upper respiratory tract disease to severe bronchiolitis and pneumonia. Serological studies documented circulation of HMPV for at least 50 years. Subsequently, this virus has been recognized as a significant cause of acute respiratory illness (ARI) in infants, children and adults Worldwide.

### Objective

To evaluate the role of HMPV in causing respiratory diseases in India.




### Achievements

HMPV is a newly discovered respiratory pathogen. In order to assess the role of HMPV in causing respiratory infections in India, specimens from 26 pediatric and 18 adult cases with acute respiratory infection from Pune, western India, were screened for the presence of HMPV RNA employing nested reverse transcriptase PCR. None of the adults and 19.2% children were positive for the viral RNA. Importantly, 4 out of 5 HMPV RNA positives were children below the age of 1 year (28.6% i.e. 4/14 children < 1 year) and one was from the age group 1-5 years (8% i.e. 1/12). Positive specimens represented both mild (n=2) and severe cases (n=3). The only fatal case was negative for HMPV RNA.

HMPV positive children included both severe and mild cases. Phylogenetic analysis based on the M gene showed grouping of the Indian HMPV isolates into two distinct genetic lineages as depected in the following figure. The results of this preliminary study demonstrated that HMPV was an important virus associated with respiratory infections in children investigated. This is the first report of HMPV cases from India.

### Legends to figure

Phylogenetic analysis of partial M gene (216 base fragment) sequences of 24 HMPV isolates.

### Future plan

The study is concluded





## 5. Standardization of *in vitro* protocols for screening of compounds for antiviral activity

**LR Yeolekar** flunetpune@yahoo.com
**VJ Lad**

Antiviral compounds are needed to reduce morbidity and economic loss due to viral infections and to treat increasing number of immunosuppressed patients who are at increased risk of infection. Researchers from government labs and private firms are increasingly requesting to provide service for evaluation of different drugs for antiviral activity.

### Objective

To standardize protocols for the evaluation of '*in vitro*' antiviral activity of pure compounds.

### Achievements

#### a. Antiviral activity of Bivalve extracts against Infectious Bronchitis virus

Lyophilized extracts of Bivalves were received from National Institute of Oceanography. Dilutions of each extract were tested for toxicity by inoculating 0.1 ml of different dilutions in eggs (4 eggs/ dilution) and observing the eggs for 5 days. Antiviral activity of each extract was tested against Infectious Bronchitis virus by titrating the virus in presence and absence of fixed dilution of extract. None of the extracts showed antiviral activity.

#### b. Standardization of antiviral activity testing against influenza A, Herpes Simplex virus type 1 and Coxsackie B3 virus.

**Influenza virus**

Different strains of influenza type A were grown in MDCK cells. All strains were screened for their ability to induce distinct cytopathic effect (CPE) in MDCK cells using 24/96 well plates. One strain of influenza type A (H1N1) (NIV # 003827) was identified and used in further tests. Amantadine hydrochloride (Sigma) at concentrations 20 to 2.5 ug/ml showed 100% plaque reduction with influenza A stock when a concentration of 42 plaque forming units (PFU)/well of A(H1N1) was used and at 3 to 0.375 ug/ml by CPE assay when one log of virus was used.

### HSV-1

HSV-1 strains (NIV # 753166 and 844036) were inoculated in Vero cells. Cells were harvested at 48 hrs when distinct CPE was observed. As a quality control measure, infected cells were probed with anti-HSV-1 and anti HSV-2 monoclonal antibodies (Chemicon) in an immunofluoresence test. Infected cells showed bright apple green fluorescence with MAbs raised against HSV-1 and did not react with MAbs raised against HSV-2 confirming that both the strains were of HSV-1 type.

Antiviral activity of acyclovir was checked by plaque assay in 24 well plates and CPE method in 96 well plates. Concentration of acyclovir showing >50% CPE inhibition or reduction in PFU compared to virus control was estimated. Preliminary results indicated that strain 753166 was resistant to acyclovir even when tested at concentration of 15 to 80 ug/ml whereas strain 844036 was inhibited at 0.3 ug/ml and above of acyclovir by both CPE and plaque assay. One

test compound under study tested at concentrations ranging from 5ug/ml to 0.3 ug/ml against HSV-1 did not exhibit antiviral activity at these concentrations.

### Coxsackie B3 virus

Strain of Coxsackie B3 (NIV # 751265) was grown in vero cells and probed with monoclonal antibodies against Cox A9, A24, B1, B2, B3, B4, B5 and B6 in IF test. Strain 751265 reacted only with MAbs against Cox B3. Testing of ribavirin for anti viral activity with this strain is in progress.

### Cytotoxicity testing

To study cytotoxic effect of compounds to be tested for antiviral activity MTT staining method was standardized. Monolayers of cells in 96 well plates were exposed to anti viral compound for 48 hrs. After 48 hrs medium was removed and 200 ul of MTT (0.75 mg/ml in medium) was added to each well. Plates were incubated at 37°C for 4 hrs. 150 ul acidified propanol (0.1 N HCL in absolute isopropanol) was added. Plates incubated at room temperature for 30 min with intermittent vigorous shaking. Plates were read at 540 nm and 690 nm.

50% cytotoxic concentration ($CC_{50}$) was defined as the concentration that reduced the absorbance by 50% as compared to control.

### Future plan

Testing of activity of candidate antiviral compounds against influenza A, HSV-1 and Cox B3 will be undertaken as a service project.





## 6. Genetic analysis of Influenza-A viruses isolated during an outbreak of encephalitis in children at Murshidabad, West Bengal, 2003.

**VA Arankalle** varanakalle@yahoo.com
**BL Rao, NS Wairagkar**

An outbreak of encephalitis during May-June 2003 in children from Murshidabad, eastern India was recently attributed to Influenza-A (H3N2). Influenza virus associated encephalopathy has been reported as a severe neurologic complication in children from Japan. It is estimated that over 100 Japanese children die every year due to Influenza virus associated encephalopathy. In the life cycle of influenza viruses, hemagglutinin (HA) and Neuraminidase (NA) proteins play crucial roles. HA is involved in the attachment of the virus to the host-cell receptors and subsequent penetration whereas NA is responsible mainly for virion release. In addition, NA is associated with penetration of the virus in the mucin layer of the respiratory tract resulting in the enhanced spread of the virus.

### Objectives

To examine if mutations in the HA and NA genes can be associated with encephalogenic potential of Influenza-A-H3N2 viruses

### Achievements

Four Influenza-A H3N2 viruses isolated during Murshidabad outbreak, two each from encephalitis and URI cases and four Influenza-A H3N2 virus strains isolated from mild respiratory cases from Pune during March-August 2003 were included in the study. HA and NA genes were amplified for all the isolates and sequenced.

### Sequence analysis

#### HA Gene

The following table gives the details of the different isolates compared in this study..

### Influenza H3N2 strains compared in this study (H Gene)

| Country | Year | Age (yr) /sex | POD | Clinical diagnosis | Specimen | Accession No | Abbreviations used |
|---|---|---|---|---|---|---|---|
| Japan* | 1998 | 4/M | 2 | Encephalitis | CSF | AB013807 | Jap/Enc/1 |
| Japan* | 1998 | 1/F | 2 | Encephalitis | CSF | AB013809 | Jap/Enc/2 |
| Japan* | 1998 | 6/M | 2 | Encephalopathy | CSF | AB013810 | Jap/Enc/3 |
| Japan* | 1998 | 1/F | 2 | Encephalopathy | CSF | AB013811 | Jap/Enc/4 |
| Japan* | 1998 | 5/M | 5 | Encephalitis | CSF | AB013812 | Jap/Enc/5 |
| Japan* | 1998 | 1/M | 6 | Encephalitis | CSF | AB013813 | Jap/Enc/6 |
| India** | 2003 | 1.5/M | 3 | Encephalitis | TS | | Ind/M/Enc/1 |
| India** | 2003 | 0.9/ | 3 | Encephalitis | TS | | Ind/M/Enc/2 |
| Japan* | 1997 | - | - | URI | TS | AB014060 | Jap/URI/1 |
| Japan* | 1997 | - | - | URI | TS | AB014061 | Jap/URI/2 |
| Japan* | 1997 | - | - | URI | TS | AB014062 | Jap/URI/3 |
| India** | 2003 | 2/M | 3 | URI | TS | | Ind/M/URI/1 |
| India** | 2003 | 4.5/M | | URI | TS | | Ind/M/URI/2 |
| India** | 2003 | 2/F | 1 | URI | TS | | Ind/P/URI/1 |
| India** | 2003 | 7/M | 2 | URI | TS | | Ind/P/URI/2 |
| India** | 2003 | 7/F | 1 | URI | TS | | Ind/P/URI/3 |
| India** | 2003 | 3M | 3 | URI | TS | | Ind/P/URI/4 |
| Zhejiang | 2003 | - | - | URI | - | - | Zhejiang/02 |
| Ningbo | 2003 | - | - | URI | - | - | Ningbo/03 |
| Johannesberg | 2003 | - | - | URI | - | - | Johannesberg/03 |
| Middleburg | 2003 | - | - | URI | - | - | Midberg/03 |
| Pretoria | 2003 | - | - | URI | - | - | Pretoria/03 |

* Mori et.al., 1999.
** Present study

In order to assess relationship of mutations in the HA and NA sequences with outcome of encphalopathy / encephalitis (E/E), Influenza-A (H3N2) viruses isolated from 2 cases each of encephalitis and URI from Murhidabad epidemic and 4 isolates from URI cases from Pune, western India (March-August, 2003) were compared with the previously reported 6 sequences from E/E cases from Japan. In addition, 6 H3N2 isolates showing maximum identity (96%-99%) with Murshidabad isolates in blast analysis were also used for comparison

The following table examines amino acid substitutions in HA1 domain as considered by Mori et. al. At nine positions, identical amino acid residues were seen irrespective of origin of the isolates. In contrast to Tyr-Phe substitution in all the 6 isolates originating from E/E cases from Japan, at position 137, all 2003 isolates exhibited Serine. Substitutions at positions 144 and 156 were mainly related to years of isolation. Similarly, the substitution at 291 (Asp-Glu) in 4/6 E/E Japanese isolates was not observed for other isolates.





### Comparative positions of deduced amino acid sequences of HA1 domain

| Virus/Year | Amino acid positions* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 121 | 124 | 133 | 137 | 142 | 144 | 156 | 158 | 194 | 196 | 226 | 276 | 291 |
| Jap/Enc/1 | N | S | N | **F** | R | I | Q | K | L | A | V | K | **D** |
| Jap/Enc/2 | N | S | N | **F** | R | I | Q | K | L | A | V | K | **D** |
| Jap/Enc/3 | N | S | N | **F** | R | I | Q | K | L | A | V | K | **E** |
| Jap/Enc/4 | N | S | N | **F** | R | I | Q | K | L | A | V | K | **E** |
| Jap/Enc/5 | N | S | N | **F** | R | I | Q | K | L | A | V | K | **E** |
| Jap/Enc/6 | N | S | N | **F** | R | I | Q | K | L | A | V | K | **E** |
| Jap/URI/1 | N | S | N | Y | R | I | Q | K | L | A | V | K | D |
| Jap/URI/2 | N | S | N | Y | R | I | Q | K | L | A | V | K | D |
| Jap/URI/3 | N | S | N | Y | R | I | Q | K | L | A | V | K | D |
| Mursh/1/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Mursh/2/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Mursh/3/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Mursh/4/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Pune/1/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Pune/2/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Pune/3/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Pune/4/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Taiwan/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Zheji/02 | N | S | N | S | R | N | Q | K | L | A | V | K | D |
| Ningbo/03 | N | S | N | S | R | N | Q | K | L | A | V | K | D |
| Johansberg/03 | N | S | N | S | R | N | **H** | K | L | A | V | K | D |
| Midberg/03 | N | S | N | S | R | N | Q | K | L | A | V | K | D |
| Pretroria/03 | N | S | N | S | R | N | Q | K | L | A | V | K | D |

* As per Mori *et al*, Arch Virol, 144, 147-55, 1999.

Eight additional substitutions were recorded in 2003 isolates. 7/8 Indian isolates showed Trp126Asp substitution as shown in the following table.

### Additional deduced amino acid substitutions

| Category | Amino acid position | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 126 | 131 | 155 | 172 | 186 | 192 | 202 | 222 | 225 |
| Japan-98 Encephalitis n=6 | W (6) | V (6) | H (6) | D (6) | S (6) | T (6) | V (6) | W (6) | G (6) |
| Japan-97 URI n=3 | W (3) | V (3) | H (3) | D (3) | S (3) | T (3) | V (3) | W (3) | G (3) |
| India-03 Encephalitis n=2 | **D** (2) | T (2) | T (2) | E (2) | G (2) | I (2) | I (2) | R (2) | D (2) |
| India-03 URI n=6 | **D** (5), W (1) | T (6) | T (6) | E (6) | G (6) | I (6) | I (6) | R (6) | D (6) |
| Other countries-03 URI n=6 | W (6) | T (6) | T (4) H (2) | E (6) | G (6) | I (6) | I (6) | R (6) | D (6) |

## NA Gene

Comparison of deduced amino acid (275 amino acids) sequences of 4 isolates from Murshidabad with other strains showing maximum nucleotide identity (94-97%) exhibited one unique substitution at position 6 (glu Lys) for all the Murshidabad isolates. However, same substitution was present in 3/4 isolates from Pune. No unique substitutions could be recorded for Murshidabad isolates.

```
#IRELAND02             TGDDENATAS FIYNGRLVDS IGSWSKKILR TQESECVCIN GTCTVVMTDG
#IND/P/URI/4           -...K..... .......... .......... .......... ..........
#IND/P/URI/3           -...K..... .......... .......... .......... ..........
#IND/P/URI/2           -...K..... .......... .......... .......... ..........
#IND/M/Enc/1           ....K..... .......... .......... .......... ..........
#IND/M/URI/1           ....K..... .......... .......... .......... ..........
#IND/M/URI/2           ....K..... .......... .......... .......... ..........
#IND/M/Enc/2           ....K..... .......... .......... .......... ..........
#DENMARK00             .......... .......... .......... .......... ..........
#VALLADOLID01          .......... .......... .......... .......... ..........
#ASUNCION01            .......... .......... .......... .......... ..........
#LYON02                ....K..... .......... .......... .......... ..........
#IND/P/URI/1           -......... .......... .V........ .......... ..........
#HONGKONG99            .......... .......... .......... .......... ..........
#NETHERLAND01          .......... .......... .......... .......... ..........
#LATIVIA00             .......... .......... .......... .......... ..........
#PERUGIA00             .......... .......... .......... .......... ..........
#MONTREAL00            .......... .......... .......... .......... ..........
#DENMARK98             .......... .......... .......... .......... ..........
#ATHENS99              ..H....... ...D...... .......... .......... ..........
#PANAMA99              ..H....... ...D...... .......... .......... ..........
#MONTEVIDEO98          ..H....... ...D...... .......... .......... ..........
#CORDOBA98             ..H....... ...D...... .......... .......... ..........
#SYDNEY97              ..H....... ...D...... .......... .......... ..........
#SANTAFE97             ..H....... ...D...... .......... .......... ..........
#SHIZUOKA97            ..H....... ...D...... .......... .......... ..........
#FUKUSHIMA96           ..H....... ...D...... .......... .......... ..........
#SHIGA97               ..H....... ...D...... .......... .......... ..........
#BUENOSAIRES96         ..H....... ...D...... .......... .......... ..........
#SOUTHAFRICA96         ..H....... ...D...... .......... .......... ..........
#NANCHANG95            ..H....... ...D...... .......... .......... ..........
#NORTHCAROLINA-SWINE98 ..H....... ...D...... ......N... .......... ..........
#TOKYO72               ..Y.K..... ...D...... .....QN... .......... ..........

#IRELAND02             SASGKADTKI LFIEEGKIVH ISTLSGSAQH VEECSCYPRY PGVRCVCRDN
#IND/P/URI/4           .......... .......... .......... .......... ..........
#IND/P/URI/3           .......... .......... .......... .......... ..........
#IND/P/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/1           .......... .......... .......... .......... ..........
#IND/M/URI/1           .......... .......... .......... .......... ..........
#IND/M/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/2           .......... .......... .......... .......... ..........
#DENMARK00             .......... .......... .......... .......... ..........
#VALLADOLID01          ...E...... .......... .......... .......... ..........
#ASUNCION01            .......... .......... .......... .......... ..........
#LYON02                .......... .......... T.L....... .......... ..........
#IND/P/URI/1           .......... .......... T......... .......... ..........
#HONGKONG99            .......... .......... T......... .......... ..........
#NETHERLAND01          .......... ...K...... T.S....... .......... ..........
#LATIVIA00             .......... .......... T.L....... .......... ..........
#PERUGIA00             .......... .......... T.L....... .......... ..........
#MONTREAL00            .......... .......... T.S....... .......... ..........
#DENMARK98             .......... .......... T.P....... .......... ..........
#ATHENS99              ....R..... .......... T.K....... .......... ..........
#PANAMA99              ....R..... .......... T.K....... .......... ..........
#MONTEVIDEO98          ....R..... ........I. ..Q....... .......... ..........
#CORDOBA98             ....R..... ........I. ..Q....... .......... ..........
#SYDNEY97              ....R..... .......... ..P....... .......... ..........
#SANTAFE97             ....R..... .......... ..P....... .......... ..........
#SHIZUOKA97            ....R..... .......... ..P....... .......... ..........
#FUKUSHIMA96           ....R..... .......... ..P....... .......... ..........
#SHIGA97               ....R..... .......... ..P....... .......... ..........
#BUENOSAIRES96         ....R..... .......... ..P....... .......... ..........
#SOUTHAFRICA96         ....R..... .......... ..P....... .......... ..I.......
#NANCHANG95            ....R..... .......... ..P....... .......... S.........
#NORTHCAROLINA-SWINE98 ....R..... .......... ..P....... .......... ..........
#TOKYO72               ....R..... .......... ..P....... .......... ....I.....

#IRELAND02             WKGSNRPIVD INVKDYSIVS SYVCSGLVGD TPRKNDSFSS SHCLDPNNEE
#IND/P/URI/4           .......... .......... .......... .......... ..........
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#IND/P/URI/3           .......... .......... .......... .......... ..........
#IND/P/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/1           .......... .......... .......... .......... ..........
#IND/M/URI/1           .......... .......... .......... .......... ..........
#IND/M/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/2           .......... .......... .......... .......... ..........
#DENMARK00             .......... .......... .......... .......... ..........
#VALLADOLID01          .......... .......... .......... .......... ..........
#ASUNCION01            .......... .......... .......... .......... ..........
#LYON02                .......... .......... .......... .......S.. ..........
#IND/P/URI/1           .......... ..I....... .......... .......S.. ..........
#HONGKONG99            .......... .......... .......... .......S.. ..........
#NETHERLAND01          .......... .......... .......... .......S.. ..........
#LATIVIA00             .......... .......... .......... .......S.. ..........
#PERUGIA00             .......... .......... .......... ......NS.. ..........
#MONTREAL00            .......... .......... .......... .......S.. ..........
#DENMARK98             .......... .......... .......... .......S.. ....N.....
#ATHENS99              .......... .......... .......... .......S.. ..........
#PANAMA99              .......... .......... .......... .......S.. ..........
#MONTEVIDEO98          .......... .......... .......... .......S.. ....N.....
#CORDOBA98             .......... .......... .......... .......S.. ....N.....
#SYDNEY97              .......... .......... .......... .......S.. ....N.....
#SANTAFE97             .......... .......... .......... .......S.. ....N.....
#SHIZUOKA97            .......... .......... .......... .......S.. ....N..D..
#FUKUSHIMA96           .......... .......... .......... .......S.. ....N..D..
#SHIGA97               .......... .......... .......... .......S.. ....N.....
#BUENOSAIRES96         .......... .......... .......... .......S.. ....N.....
#SOUTHAFRICA96         .......... .......... .......... .......S.. ....N.....
#NANCHANG95            .......... .......... .......... .......S.. ....N.....
#NORTHCAROLINA-SWINE98 .......... .......... .......... ......RS.. .Y..N.....
#TOKYO72               .......V.. ........D. .......... ...N..RS.N .Y.RN....K

#IRELAND02             GGHGVKGWAF DDGNDVWMGR TISEKLRSGY ETFKVIEGWS KPNSKLQINR
#IND/P/URI/4           .......... .......... .......... .......... ..........
#IND/P/URI/3           .......... .......... .......... .......... ..........
#IND/P/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/1           .......... .......... .......... .......... ..........
#IND/M/URI/1           .......... .......... .......... .......... ..........
#IND/M/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/2           .......... .......... .......... .......... ..........
#DENMARK00             .......... .......... .......... .......... ..........
#VALLADOLID01          .......... .......... .......... .......... ..........
#ASUNCION01            .......... .......... .....S.... .......... ..........
#LYON02                .......... .......... .......... .......... ..........
#IND/P/URI/1           .......... .......... .......... .......... N.........
#HONGKONG99            .......... .......... .......... .......... ..........
#NETHERLAND01          .......... .......... .......... .......... .A........
#LATIVIA00             .......... .......... .......... .......... ..........
#PERUGIA00             .......... .......... .......... .......... ..........
#MONTREAL00            .......... .......... .......... .......... ..........
#DENMARK98             .......... .......... .......... .......... ..........
#ATHENS99              .......... .......... .....S.... .......... ..........
#PANAMA99              .......... .......... .....S.... .......... ..........
#MONTEVIDEO98          .......... .......... .....F.... .......... ..........
#CORDOBA98             .......... .......... .....F.... .......... ..........
#SYDNEY97              .......... .......... .....F.... .......... ..........
#SANTAFE97             .......... .......... .....F.... .......... ..........
#SHIZUOKA97            .......... .......... .......... .......... ..........
#FUKUSHIMA96           .......... .......... .......... .......... ..........
#SHIGA97               .......... .......... .......... .......... ..........
#BUENOSAIRES96         .......... .......... .......... .......... ..........
#SOUTHAFRICA96         .......... .......... .......... .......... ..........
#NANCHANG95            .......... .......... .......... ......G... ..........
#NORTHCAROLINA-SWINE98 .......... .......... .......... ......G... ..........
#TOKYO72               .N........ .......... ....DS.... ......G... T.........

#IRELAND02             QVIVERGNRS GYSGIFSVEG KSCINRCFYV ELIRGRKEET EVWWTSNSIV
#IND/P/URI/4           .......... .......I.. .......... .......... ..........
#IND/P/URI/3           .......... .......... .......... .......... ..........
#IND/P/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/1           .......... .......... .......... .......... ..........
#IND/M/URI/1           .......... .......... .......... .......... ..........
#IND/M/URI/2           .......... .......... .......... .......... ..........
#IND/M/Enc/2           .......... .......... .......... .......... ..........
#DENMARK00             .......... .......... .......... .......... ..........
#VALLADOLID01          .......... .......... .......... .......... ..........
#ASUNCION01            .......... .......... .......... .......... ..........
#LYON02                ....D..... .......... .......... ......NQ.. ..L.......
#IND/P/URI/1           ....D..... .......... .......... .......Q.. ..L.......
#HONGKONG99            ....D..... .......... .......... .......Q.. ..L.......
#NETHERLAND01          ....D..... .........S .......... .......Q.. ..L.......
#LATIVIA00             ....D..... .......... .......... .......Q.. ..L.......
```

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#PERUGIA00              ....D..... .......... .......... .......Q.. ..L.......
#MONTREAL00             ....D..... .......... .......... .......Q.. ..L.......
#DENMARK98              ....D..... .......... .......... .......Q.. ..L.......
#ATHENS99               ........M. .......... .......... .......Q.. ..........
#PANAMA99               ........M. .......... .......... .......Q.. ..........
#MONTEVIDEO98           ....D.D... .......... .......... .......Q.. ..........
#CORDOBA98              ....D.D... .......... .......... .......Q.. ..........
#SYDNEY97               ....D..... .......... .......... .......Q.. ..........
#SANTAFE97              ....D..... .......... .......... .......Q.. ..........
#SHIZUOKA97             ....D..... .......... .......... .......Q.. ..........
#FUKUSHIMA96            ....D..... .......... .......... .......Q.. ..........
#SHIGA97                ....D..... .......... .......... .......Q.. ..........
#BUENOSAIRES96          ....D..... .......... .......... .......Q.. ..........
#SOUTHAFRICA96          ....D..... .......... .......... .......Q.. ..........
#NANCHANG95             ....D..... .......... .......... .......Q.. ..........
#NORTHCAROLINA-SWINE98  ....D..... .......... .......... .......Q.. ..........
#TOKYO72                ....DSD... .......... .......... ......EQ.. R.........

#IRELAND02              VFCGTSGTYG TGSWPDGADI NLMPIAFAIL E------
#IND/P/URI/4            .......... .......... ....------ -------
#IND/P/URI/3            .......... .......... ....------ -------
#IND/P/URI/2            .......... .......... ....------ -------
#IND/M/Enc/1            .......... .......... .....----- -------
#IND/M/URI/1            .......... .......... .....----- -------
#IND/M/URI/2            .......... .......... .....----- -------
#IND/M/Enc/2            .......... .......... .....----- -------
#DENMARK00              ......R... .......... .........-- -------
#VALLADOLID01           .......... .......... .......... -------
#ASUNCION01             .......... ....------ ---------- -------
#LYON02                 .......... .......... .......... .------
#IND/P/URI/1            .......... ........E. ....------ -------
#HONGKONG99             .......... .......... ..........KTPCFY
#NETHERLAND01           .......... .......... .......... -------
#LATIVIA00              .......... .......... ..........KTPCFY
#PERUGIA00              .......... .......... ..........KTPCFY
#MONTREAL00             .......... .......... .....----- -------
#DENMARK98              .......... .......... ..........KTPCFY
#ATHENS99               .......... .......... .........- -------
#PANAMA99               .......... .......... .......... .------
#MONTEVIDEO98           .......... .......... ..........KTPCFY
#CORDOBA98              .......... .......... ..........KTPCFY
#SYDNEY97               .......... .......... ..........KTPCFY
#SANTAFE97              A......... .......... ..........KTPCFY
#SHIZUOKA97             .......... .......... .....----- -------
#FUKUSHIMA96            .......... .......... .....----- -------
#SHIGA97                .......... .......... .....----- -------
#BUENOSAIRES96          .......... .......... ..........KTPCFY
#SOUTHAFRICA96          .......... .......... ..........KTPCFY
#NANCHANG95             .......... .......... ..........KTPCFY
#NORTHCAROLINA-SWINE98  .......... .......... ---------- -------

#TOKYO72                .......... .......... .....----- -------
```

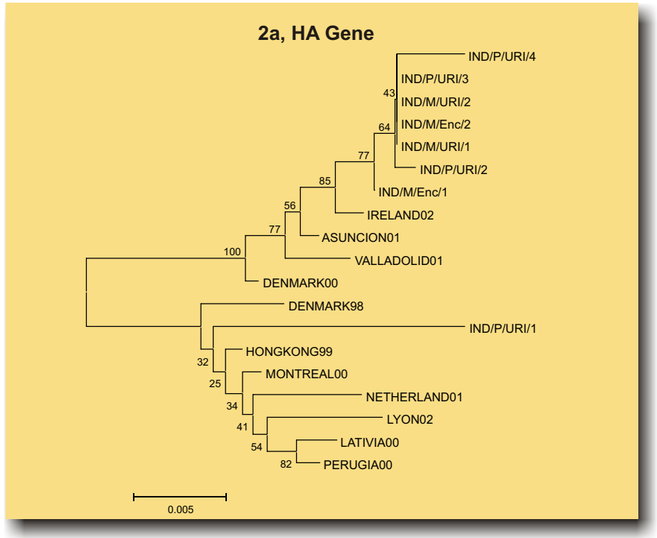
Partial NA gene amino acid sequence alignment

## Phylogenetic analysis

To elucidate observed variability among HA and NA genes isolated during 2003, these genes were further analyzed phylogenetically. As evident from figure 2a, isolates from encephalitis cases from Japan (1998) and India (2003) clustered separately alongwith the other strains circulating at the same time. Overall, within the Japanese and Indian patients 99.6% identity at nucleotide level was noted. Irrespective of the source of isolation, Japanese strains were 96.6-97% identical with the Indian strains. NA gene-based phylogenetic analysis (Figure 2b) showed that all the 4 isolates from Murshidabad (June 2003) and 3 of the 4 Pune isolates (July-August 2003) grouped together whereas the Pune isolate recovered in March 2003 (IND/P/URI/1) grouped separately. Thus, phylogenetic analyses based on both HA and NA genes showed that IND/P/URI/1 isolate was divergent from the remaining Indian viruses isolated during June-August 2003.








Phylogenetic analyses of HA gene (876 nucleotides, figure 2a) and NA gene (831 nucleotides, figure 2b) of Influenza-A H3N2 isolates.

## 1. Molecular surveillance of Measles and subacutesclerosing panencephalitis (SSPE) in India from 2003

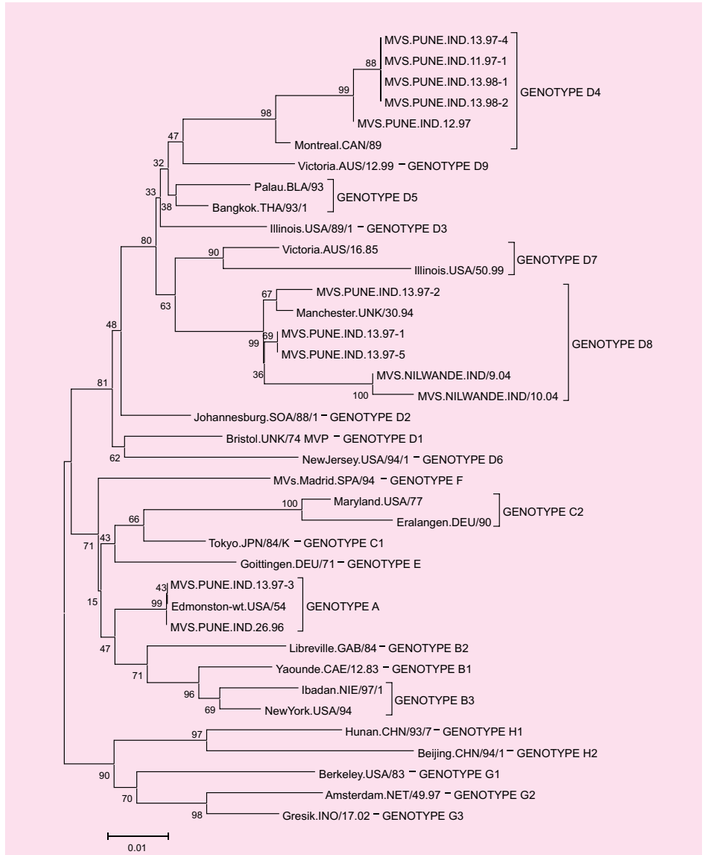
**NS Wairagkar** niteen@hotmail.com
**SR Vaidya**

### Objectives

Molecular genetic characterization of measles virus strain from India.

### Achievements

Two Indian strains of measles viruses were phylogenetically analysed and shown to be genotye D8.




### Future proposals

Following Extramural Project "Genotyping of Measles virus strains circulating in various parts of India" is submitted to WHO Initiative on Vaccine Research (IVR) for funding.





# Measles

# Other Viruses

## 1. Studies on Human papillomavirus (HPV), Epstein-Barr virus (EBV), herpes simplex virus (HSV) infections in Oral cancers.

V Gopalkrishna gopalvk58@hotmail.com

Human papillomaviruses and Epstein Barr viruses have been found to be associated in upper aero digestive tract lesions including oral cancers. HPV 16 and 18 DNA are considered as “high risk” types and 6 and 11 as “low risk” factors in the development of such malignancies. Synergistic roles of these in producing oncogenic lesions remained incompletely understood. The present study examined the prevalence of HPV and EBV in case of oral cancers.

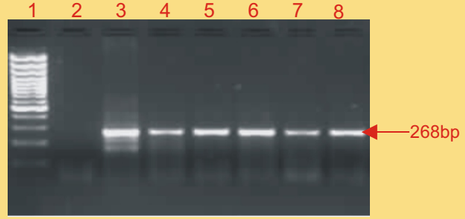
### Objectives

Detection of human Papillomavirus types associated with oral malignancies.

### Achievements

During the study period, a total of forty-five surgically operated oral cancer tissue specimens were obtained from local collaborating hospitals of NIV, Pune for the detection of HPV, EBV DNA by polymerase chain reaction (PCR). Cancer cell lines such as SiHa, Hela and Raji were used as positive controls. All the DNA samples isolated from the tumor tissues were initially screened for â-globin gene, which served as internal control. A total of 42.2% (n=45) of HPV DNA positivity was established in oral cancer patients. When type specific HPV primers were used, HPV type 16 was the most predominant type found in 14 out of 45 (31.2%) of the patients. HPV 18 was detected only in 6.6% and HPV 6 and HPV 11 in 2.2% (n=45) of the cases respectively. A high prevalence of EBV DNA positivity i.e. 44.5% (n=45) was also observed by PCR when primers of BamHI-W repeated region of EBV genome used. However, primers of BNLF-1 transforming region of EBV were used, only 13.3% of the cases showed positivity. Interestingly, co-infections of HPV genotypes and EBV were also observed. EBV DNA (BNLF-1) positivity could also be observed in HPV negative oral cases. Normal control samples showed only 10-13% positivity of either of the viruses studied. All the patients were having tobacco chewing/ brushing habits for more than 15 years duration.

**PCR Amplification of β-globin gene in oral cancer specimen**

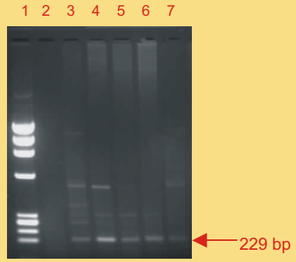



Lane 1 : 100 bp ladder
Lane 2 : Negative control
Lanes 3-8 : Oral cancer DNA amplified with β globin gene



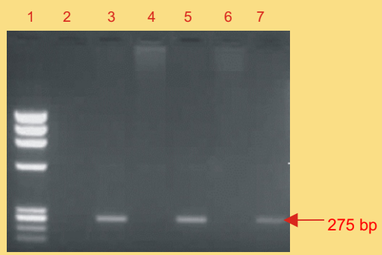


**Detection of EBV DNA (BamHI-W) in oral cancer patient**




**Lane 1: Φx DNA HaeIII digest**
**Lane 2: Negative control**
**Lanes 3-8: Oral cancer DNA samples Positive for EBV**

**PCR amplification of EBV(BNLF-1) sgene in oral specimens**




**Lane 1: Φx DNA HaeIII digest**
**Lane 2: Negative control**
**Lanes 3-8: Oral cancer DNA samples Positive for EBV**

### Future plan

The study will be continued. Individual genotyping of high risk HPV's will be carried out by using type specific viral primers.

## 2. Human papillomavirus infection in HIV infected women attending STD clinics and its correlation with cervical abnormalities

**V Gopalkrishna** gopalvk58@hotmail.com
**SN Joshi, S Mehendale (NARI)**

Human Papillomaviruses have been implicated in the development of cancer of the cervix. “High risk” HPV type 16 and 18 are considered as potential risk factors during progression of the disease. Recently, HIV infection and related immunosuppression are found to be associated with excess risk for cervical neoplasia with persistence HPV infections. The present study examines the prevalence of HPV in HIV infected cases.

### Objectives

- To detect the prevalence of HPV infections in HIV infected women with cervical abnormalities.
- To characterize HPV genotypes associated in HIV infected women with cervical abnormalities.
- To correlate the viral infections with clinical and other epidemiological risk factors.

### Achievements

During the present study period, a total of 100 cervical swabs collected from the HIV infected women were subjected to DNA extraction and HPV study. Cytological diagnosis of the cervical abnormalities was carried out by using 'PAP' test. Polymerase chain reaction (PCR) method was used to detect HPV DNA and also genotyping of HPV's. 'PAP' smear abnormalities were more commonly observed among women aged 30 yrs and above (P=0.035,OR 2.8, 95% CI 1.04-7.8). Of the 100 randomly selected cervical swabs screened, HPV 16 and 18 DNA were detected in 33% (95% CI 23.9-43.1) of the cases. HPV 16 was the predominant type found and dual infection of both 16 and 18 were also observed in one case. HPV 16 and 18 were present in 28.3% of women below 30 years of age and 40.5% women aged above 30 years. There was no statistically significant difference observed between presence of HPV (16 and 18), ART, CD4 cell count and parity of the women.

### Future plan

The present study will be continued to analyze more number of HIV infected women with various cervical abnormalities. “High risk” HPV patient group will be constantly monitored and regularly followed up for any progression of higher lesions.





## 3. Surveillance of Acute Flaccid Paralysis (AFP) cases from Karnataka State as a part of WHO-SEAR Polio Lab Network.

**PN Yergolkar** nivbng@bgl.vsnl.net.in

Laboratory Surveillance work for Global Eradication of Poliomyelitis programme was initiated by the Govt. of India in collaboration with the WHO/WHO-SEARO/WHO-NPSP in India since June 1997. NIV Field Station, Bangalore is responsible for processing all the stool specimens from Karnataka State.

### Objectives

- To isolate and identify wild polioviruses from AFP cases.
- To participate as one of the 8 National Polio Laboratories in India in the WHO-SEAR Polio Lab Network activities.
- To refer all polio virus isolates to ERC, Mumbai for intratypic differentiation tests.
- To fulfil 7 criteria of WHO for annual laboratory accreditation by the WHO to be part of the WHO-SEAR Polio Lab Network.

### Achievements

During the period from April 2003 to March 2004, a total of 905 stool specimens were received from 452 AFP cases (data as on 29-04-04). Of these, 427 cases were from Karnataka state, 14 from Andhra Pradesh, 4 each from Maharashtra and Tamil Nadu and 3 from Kerala. AFP cases other than Karnataka State had onset in respective states and were investigated in Karnataka. Fortyone P1 Wild type, 24 Polio Vaccine, 120 non-polio enteroviruses (NPEV) only, were isolated. 264 were negative. Of 24 polio vaccine isolates, three were P1, four were P2, nine were P3, one was P1+P2, three were P1+P3, two were P2+P3 and remaining two were P1+P2+P3.

Of the 41 P1 wild cases, 37 were from Karnataka and 4 were from Andhra Pradesh. Previous last wild polio case was in December 2000 in Karnataka and December 1999 in Andhra Pradesh. Wild Polio-1 cases were reported from Bellary districts, after a gap of 2 years and 4 months. District wise cases in Karnataka were, Bellary-18, Raichur-8, Koppal-4, Gulbarga and Bagalkot 3 each and Chitradurga-1. Of the 21 wild P1 virus isolations from Andhra Pradesh four were isolated at NIV, Bangalore and 17 were isolated at King Institute of Preventive Medicine, Chennai.

### Month-wise Results of AFP cases

| Month | No. of AFP cases tested | Polio-1 Wild | Polio Vaccine | NPEV only | Negative |
|---|---|---|---|---|---|
| April - 2003 | 34 | 0 | 4 | 5 | 25 |
| May | 26 | 1 | - | 7 | 18 |
| June | 30 | 3 | 1 | 11 | 15 |
| July | 50 | 11 | - | 16 | 23 |
| August | 71 | 9 | 3 | 28 | 31 |
| September | 48 | 9 | 2 | 14 | 23 |
| October | 43 | 2 | 2 | 10 | 29 |
| November | 35 | 3 | - | 7 | 25 |
| December | 22 | 2 | 2 | 7 | 11 |
| January - 2004 | 30 | - | 4 | 4 | 22 |
| February | 35 | 1 | 6 | 3 | 25 |
| March | 28 | - | - | 8 | 17 |
| Total | 452* | 41 | 24 | 120 | 264 |

AFP cases were reported from both the sexes and in all age groups upto 15 years. OPV immunization status among 41 wild polio cases was '0'-dose in 17, 1-3 doses in 19 and 4+ doses in 15.

### Age-Sex distribution of AFP cases

| Age group in years | Male | Female | Total |
|---|---|---|---|
| < 1 | 28 | 23 | 51 |
| 1-2 | 87 | 66 | 153 |
| 3-5 | 81 | 41 | 122 |
| 6-10 | 66 | 24 | 90 |
| 11-15 | 19 | 17 | 36 |
| **Total** | **281** | **171** | **452** |

### Age group wise Results of AFP cases

| Age Group in Years | No. of AFP cases tested | Polio Wild (P1) | Polio Vaccine | NPEV only | Negative |
|---|---|---|---|---|---|
| < - 1 | 51 | 9 | 2 | 13 | 27 |
| 1 - 2 | 153* | 25 | 12 | 56 | 57 |
| 3 - 5 | 122 | 6 | 8 | 34 | 74 |
| 6 - 10 | 90 | 1 | 2 | 16 | 71 |
| 11 - 15 | 36 | - | - | 1 | 35 |
| **Total** | **452*** | **41** | **24** | **120** | **264** |

During the period, 15 stool specimens from healthy contacts of 3 index AFP cases were tested. One P1 Wild, 5 NPEV only were isolated. One P1-wild virus isolated from contact was from 2 years female child from Gulbarga city in the month of August and the child had 1 dose of OPV only.

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

Onsite review visit for WHO-Accreditation of the Polio Laboratory was held on 1-3 April 2004, by the WHO-SEAR Polio Laboratory Network (Review period 1st January to 31st December 2003).





### Criteria for accreditation and targets achieved

| Target | Achievement |
|---|---|
| Tests are performed on atleast 150 Stool specimens annually | 1003 stool specimens tested - 964 from AFP cases and 99 from Healthy Contact cases |
| Score on annual onsite review is atleast 80% | Onsite review Score is 99% in April 2004 visit |
| Test results on 80% of all AFP specimens are reported within 28 days | 100% - 1003/1003 reported ≤ 28 days. |
| Accuracy of poliovirus typing is atleast 90% | 100% - 157/157 Polio isolates reconfirmed |
| Atleast 80% of poliovirus isolates from AFP cases to be forwarded for intratypic differentiation within 7 days | 100% - Polio isolates sent within 7 days. |
| Results on most recent Proficiency Test Panel is atleast 80% | 100% Jan 2003 & Feb 2004 |
| Internal Quality Control procedures are implemented atleast Quarterly | Implemented internal quality control procedures quarterly |

## Conclusion

Wild poliovirus-1 activity in 6 districts of Karnataka after a gap of 2 years was confirmed with 37 wild P1 isolations from AFP cases. Wild polio was also detected from Andhra Pradesh after a gap of 3 years. Wild P3 was not detected for last 3-4 years.

## Future plan

There is continued need of AFP surveillance until Global Eradication & Certification process as per the Global decision of the WHO and Govt. of India, as per programme conditions.

## 4. Prevalence of simian immunodeficiency virus (SIV) and simian retroviruses (SRV) in wild caught captive simian population.

**AC Mishra** acm1750@rediffmail.com
**VA Poddar**

Interest in the study of SIV was sparked by the observation that though the SIV could infect many African monkeys without causing disease, it could cause an AIDS like disease SAIDS in the Asian macaques and some Old World Monkeys. Most of the SIV/SRV studies concentrated on animals in various regions of tropical Africa. The first report was published in 1998, on SIV from south and east Africa reporting an $SIV_{agm}$ variant from Chacma baboon. Thus so far no report on SIV infection has been published from India. Additionally there are no reports from India regarding prevalence of simian retroviruses (SRV), which also cause SAIDS except our previous report of SRV-6 from Hanuman langur.

### Objectives

To study the natural infection by retroviruses (SIV/SRV) in captive non-human primate from different animal house facilities.

### Achievements

Study was carried out for identification of a related SRV-6 naturally infecting the captive population of rhesus monkeys at CDRI Lucknow. Total 5 sera, 15 stool samples and 10 PBMC were collected. Serology and molecular characterization was done for SIV/SRV. All sera were screened by anti HIV1 and anti hiv2 commercial rapid and ELISA kits. Serologically all samples were negative for anti HIV Ab. Western blot was done for all 25 sera and three samples were found weakly reactive showing band corresponding to p24. Further confirmation of WB was done by RT-PCR for env region (gp 20 region of SRV) and for gag region of Siv. Samples were negative for SIV and SRV. Antibody and genomic detection of SIV/SRV from 15 stool samples was negative.

### Conclusion

SRV 6 virus was not detected in captive monkeys from Lucknow.

## 5. Virological and Immunologcal studies of Lymphadenopathy in HIV Infected patients.

**AC Mishra** acm1750@rediffmail.com
**SS Tikute**

In HIV infected patients, lymphadenopathy is a common manifestation. According to recent report, from Memorial Slaon Ketering Cancer Center, USA, the incidence of Non Hodgkin's lymphoma (NHL) is over hundred times increased and Hodgkin's disease (HD) over ten times increased in HIV infected population. Majority of HIV related HDs are linked to Epstein Barr virus, which ranges from 80 to 100%. A study on clinical profile of AIDS in India suggests 22.5 to 28% HIV patients had generalized lymphadenopathy. In other studies of malignancies associated with HIV infection, lymphoma has been reported from India.

### Objectives

To investigate association of different viruses like HIV, HTLV, EBV, HHV and HRV-5 in AIDS associated lymphadenopathy.

### Achievement

Extension of work on this project is going on. Nine paired samples of FNAC's and Blood is taken from virology OPD of Sassoon hospital. Serology for HIV is done. FNAC's culture is done. Co-culture is done with H-9 and Raji cells, supernatant are stored at -70 degrees. Infected cells are stored in Liquid Nitrogen.

## 6. Studies on Ganjam virus around Pune.

**MV Joshi** **mvjoshi46@rediffmail.com**

Ganjam virus has been isolated earlier from *Haemaphysalis intermedia* (*H. intermedia)* ticks collected off domestic animals from Orissa, Andhra Pradesh and Karnataka state. Since no data is available on the prevalence of tick borne viruses in and around Pune, attempts were made to isolate viruses from ticks collected off domestic animals from certain localities around Pune during 2001-2002.

Tick collection by flag dragging

### Objectives

Isolation, identification and characterization of tick- borne Ganjam viruses isolated from ticks.

### Achievement

Ten virus isolates were obtained from119 pools of ticks comprising 1138 adult ticks collected off domestic animals around Pune. Six of the ten isolates were identified as Ganjam virus by Quick CF test and confirmed by RT-PCR. The remaining four isolates were reinoculated in Swiss albino mice and further serial passages were carried out. Brains of sick mice were harvested and stored at $70^{o}$C. Further work on identification of the isolates is in progress.

### Preparation of virus pool and immune serum

Ganjam virus (G619) pool was prepared in 2 3 day-old infant Swiss albino mice and titrated (titre$10^{5}$). The virus pool was lyophilised and stored at $20^{0}$C. Immune serum against Ganjam virus was also prepared in mice and titrated in CF test. The Ganjam virus isolates have been lyophilised and stored at $20^{0}$C for molecular characterization studies.

### Future plan

Studies on prevalence of antibodies to Ganjam and other tick borne viruses in humans and animals will be undertaken in the same areas from where Ganjam virus isolates have been obtained recently.

## 7. Development of HIV-1 C subtype vaccine candidate using SFV-replicon strategy. (ICMR-IAVI-BIOPTION collaboration)

**Cecilia D** cdayaraj@hotmail com

An Indian subtype C isolate had been selected for designing the SFV based vaccine. The gag, pol nef, rev and tat genes were synthesized with several mutations incorporated to inactivate the expressed proteins.

### Objective

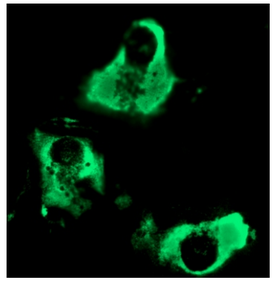
Development of a SFV replicon based vaccine for HIV-1 subtype C using an Indian strain

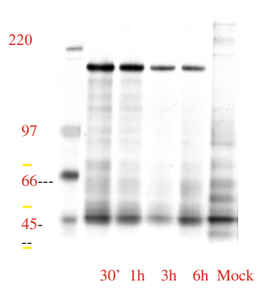
### Work Plan

- The gag/pol/nef genes were cloned into the SFV expression vector
- The rev/tat was cloned into another construct.
- Recombinant particles were produced by transfection of cells with *in vitro* transcribed RNA from the recombinant plasmid and the helper plasmids expressing spike and capsid proteins.

### Achievements

The gag/pol/nef (5 kb), the rev/tat with both exons (682 bp) and rev/tat with only exon1 were cloned into the pSFV vector. All constructs expressed well as determined by metabolic labelling followed by SDS-PAGE and by Immunofluorescence assay. All the antigens localized to the cytosol and were reactive with the specific antibodies. The titres of the particles, packaged with the three different vectors were high, demonstrating that even the 5 kb large insert was well tolerated in the vector.






Inactivation of the genes was confirmed by the observation that the gag/pol/nef protein was expressed as a single polypeptide (no fragments were observed indicating lack of protease activity) and the expressed proteins did not localize to the nucleus (lack of nuclear localization signals). No reverse transcriptase activity was observed in the RT assay performed using lysates from cells infected with SFV particles expressing the gag/pol/nef gene. The function





of tat gene was tested in GHOST cells, obtained from the AIDS repository. The EGFP reporter gene, under the HIV promoter, is transactivated in these cells by Tat protein. GHOST cells infected with pSFV20E-rev/tat particles did not show expression of EGFP indicating that the expressed tat was functionally inactive.

The immunogenicity of the recombinant particles was recently shown in mice by the lab in Sweden.

### Future plan

The development of the vaccine is ongoing at Bioption, Sweden.

## 8. Studies on ultrastructure of some novel viruses isolated from India

**Atanu Basu** atanu_b@Hotmail.com

NIV has isolated novel viruses from diverse species, during both routine arbovirus surveillance and outbreak investigation studies. The present report highlights the data from studies on ultrastructure of Chittoor virus.

The Chittoor virus (CV) was first isolated from *Anopheles barbirostris* mosquitoes in Chitoor district of Andhra Pradesh, a state in southern India in 1957. Subsequently, the virus was seen to be closely related to the Batai virus and was described as a Bunyavirus. Antibodies to CV were reported in different species of animals (mainly domestic ungulates) and humans in different states of India.

### Objective

Study the ultrastructure of the Chittoor virus by electron microscopy.

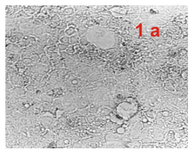
### Achievements

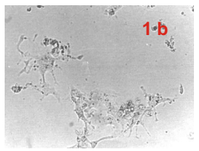
#### Cytopathic effect on cell lines

Distinct CPE was observed in both Vero and Neuro 2A cells on the 2$^{nd}$ post inoculation day when compared with uninfected controls. No CPE was seen in the RD cells observed upto a week after infection. In the vero cells the CPE pattern was consistently observed as grouping of cells that degenerated rapidly. In the neuro 2A cells, the cellular degeneration pattern was also rapid involving most cells and seen in the form of rounding and floating cells. Interestingly, no CPE-like changes could be observed on the RD cell line inoculated with the Chittoor virus upto 5$^{th}$ day post-inoculation.

### Cytopathic effect of Chittoor virus in different cell lines

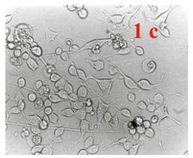
Uninfected vero cells

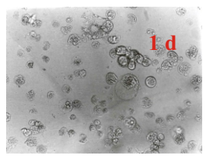



Infected vero cells showing CPE

Uninfected neuro 2A cells



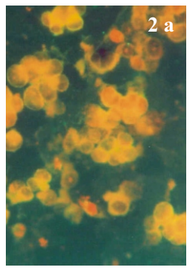




Infected neuro 2A cells showing CPE

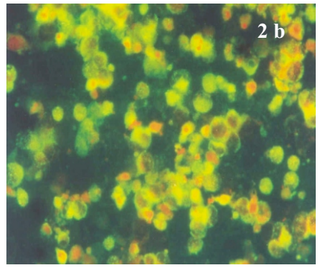
### Distribution of viral antigens by IFA & flowcytometry

The pattern of viral antigen distribution in the infected vero cells on the 3rd p.i.d as seen from the confocal microscopy imaging, was distinctly within the cytoplasm in a punctate form. In some cells areas of perinuclear fluorescence could also be detected. No nuclear fluorescence was observed. Flowcytometric analysis of the infected cells showed approximately 77 % cells expressing viral antigens.

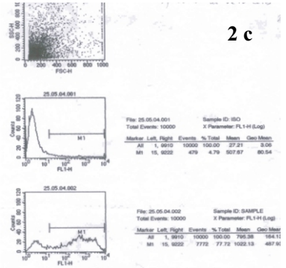
### Detection of Chittoor virus antigens in infected cells by IFA and flowcytometry




2a: Uninfected vero cells




2b: Chittoorvirus infected vero cells 4th PID




Flowcytometry of infected vero cells

### Ultrastructure of the Chittoor Virus

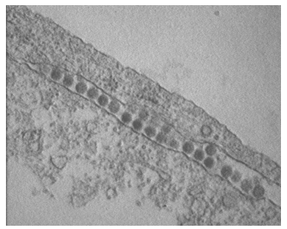
Examination of culture fluids from CPE+ve cultures in negative stained preparations, showed distinct presence of a *Bunyavirus*, partly pleomorphic with size range of 70-90 nm and an envelope structure not easily definable (figure 3). Examination of ultrathin sections of the infected cells showed distinct presence of cell associated virus particles in different stages of morphogenesis. Virus particles were seen to be associated with the cell membrane and early entry was detected in association with coated vesicles. Replication of the virus was seen in the cytoplasm as electron-dense “viroplasm” and mature virions could be seen in the golgi, ER and cytoplasmic vesicles. No inclusion bodies could be detected in the cells examined.



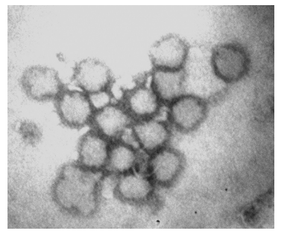


No cells with apoptotic ultrastructure could be seen. Most of the maturation sites for this virus could be imaged within the secretory ergastroplasmic mass of the infected cells. Interestingly, no maturation of viruses directly at the cytoplasmic membrane could be observed. However, in one field a group of mature virions could be seen “egressed” from the cytoplasm of an infected vero cell. These observations are the first electron microscopic description of the Chitoor (Batai?) virus, a pattern consistent with typical morphogenesis of a *Bunyavirus*.

### Ultrastructure of the Chittoor virus



Transmission electron micrograph of an infected vero cell showing arrays of virus particles in cytoplasmic lamellar-structure near the cell membrane. Most particles have morphology of intact virions, 70-90 nm in size. The structure is most likely golgi-derived. Bar= 100 nm



Negative stained transmission electron micrograph of Chittoor virus particles in culture supernatant from infected vero cells showing CPE

## 9. Isolation and partial characterization of a pestivirus from autopsied goat specimen from Tamil Nadu, India.

**DT Mourya** mouryadt@vsnl.net
**MV Joshi, P Yadav**

In 1994 there was a disease outbreak in Tamil Nadu, southern India, affecting sheep and lamb with clinical features resembling that of Rift Valley Fever Virus (RFV) like illness. The etiologic agent was not identified.

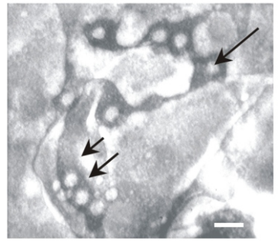
### Objective

Virus isolation studies from autopsied tissue of a lamb from the 1994 RFV-like illness.

### Achievements

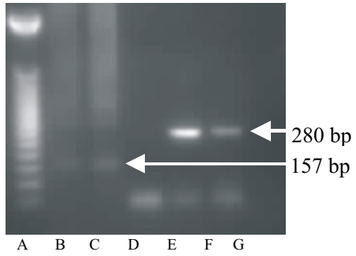
Inoculation of the clarified autopsied-spleen specimen from the lamb suspected to have died of the RFV-like illness in 1994 was inoculated into suckling mice brain. Acute sickness was observed in the mice and mice brain passaged into cell lines (vero, RD) showed evidence of cytopathic effect on the 3rd day after infection. Electron microscopy of the culture fluid from the CPE (+ve) cultures showed presence of flavivirus-like particles , 30-40 nm in size. Subsequent PCR analysis confirmed the isolate as Bovine Diarrheal Disease Virus (BVDV).

**Electron micrograph showing flavivirus like particles in negative staining preparation**




**Agarose gel showing RT-PCR-assays for Pestivirus in the RD cell line and sick mice brain infected with virus**

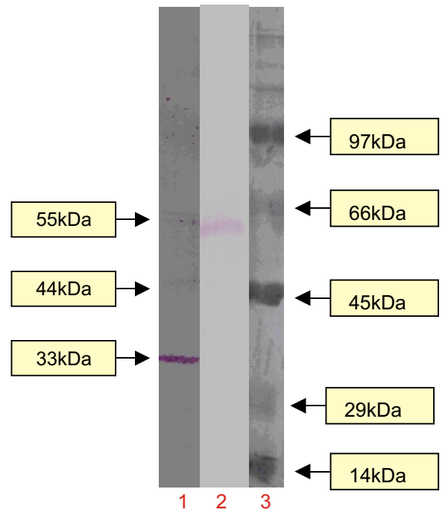
Size marker, 50 bp ladder (lane A); Infected RD cell line nested PCR (lane B); Infected sick mice brain nested PCR (lane C); Uninfected mice brain as negative control (lane D); Infected RD cell line first PCR (lane E); Infected sick mice brain first PCR (lane F); mice brain as negative control (lane G).








**Western-blot analysis of BVDV virus stock, probed with HX-2 MAb**
cross reactive to E protein of flaviviruses




Lane-1: Ultra-centrifuged suspension pellet from BVDV infected Vero cell lyset
Lane-2: Ultra-centrifuged suspension pellet from dengue infected Vero cell lyset
Lane-3: Molecular weight markers.

### Future plan

Studies concluded.

National Institute of Virology
Miscelleneous Studies

# Miscelleneous Studies





Annual Report 2003-04

## 1. Isolation of CHP virus from blood clots received from Karimnagar, AP.

As a combined effort to identify the causative agent of outbreak of encephalitis in Andhra Pradesh and Maharashtra samples attempts to isolate the virus from earlier standardized method were made.

Blood clots from ten patients were subjected to ammonium chloride treatment to lyse RBCs. WBC collected were co cultured with phytohemaglutinin stimulated peripheral blood mononuclear cells from normal individuals and incubated for two days. After two days all the cultures were further passaged twice in PBMC cultures. Culture supernatant fluid was examined by electron microscopy. Two isolates could be obtained which were confirmed as CHP virus by PCR assay.

## 2. Standardization of microtitre neutralization test for detection of neutralizing antibodies to CHP virus

Microtitre neutralization test was standardized by using Vero cell passaged CHP virus. Using this test more than 300 sera were assayed for the presence of neutralizing antibody response against CHP virus. Results of these tests are reported under the human and animal serological response to CHP virus.

## 3. Development of Monoclonal antibodies against CHP virus.

Spleenocytes from CHP virus immune BALB/c mice were fused with SP2/0 cells. Hybrids obtained were selected using virus-neutralizing assay. This insured that MAbs against nonneutralizing and cross-reactive nucleoprotein were not obtained. Seven MAbs could be generated having neutralizing activity against CHP virus. Titres of the MAb tissue cultures ranged from 1:4 to 1: 63000. None of the antibodies neutralize closely related vesicular stomatitis virus. These antibodies have been used for developing antigen capture ELISA successfully.

## 4. Attempts to isolate etiologic agent from samples of encephalitis cases from Saharanpur, UP.

Attempts were made to isolate the etiological agent from samples obtained from encephalitis cases from Saharanpur UP. Passaging of PBMCs, and mosquito suspension in mice and PBMC cultures have been attempted. Although sickness in mice has been recorded isolation and characterization of any etiological agent / factor has not yet been identified. Attempts are continuing.

## 5. Experimental transmission of four strains of WN virus by *Aedes albopictus* mosquitoes

In India, West Nile virus enzootic cycle is silent and the occurrence of deaths in birds has not been noticed as well as it has not been recorded in epidemic form. Where as in the United States and other industrialized nations disease strikes urban areas and *Ae. albopictus* is incriminated as one of the important vector. In India role of this mosquito species is not known. Therefore, study was conducted to determine WN virus transmission capabilities of *Ae. albopictus* mosquito. Results showed that orally infected mosquitoes of this species are capable of transmitting efficiently all the four strains of virus (B-04994, E101, G-2288 and P-4230) to infant mice.





## 6. Epidemic investigation of Febrile illness

An epidemic of febrile-illness was reported from Kerala state during July, 2003. Epidemic investigations were carried out by NIV jointly with CRME, Madurai unit. People living in the rubber plantation areas were mainly affected. Entomological investigations showed that there were very few *Aedes aegypti* mosquito but density of *Aedes albopictus* mosquito was very high. Due to heavy rains, rubber-collection containers were filled with water and there was massive breeding of *Ae. albopictus* mosquito in large number of such pots. Patient's sera were inoculated in infant Swiss Albino mice. Brain of sick mice subjected to RT-PCR showed the presence of DEN-2 and DEN-4 viral RNA.

## 7. Concurrent multiple dengue serotype infection

Serum samples from the suspected dengue cases from Maharashtra, Gujarat and Kerala collected in 2003 were referred to this institute for laboratory diagnosis. Samples were tested by indigenously developed ELISA kits, which confirmed the recent dengue virus infection (IgM) in at least 30% of cases. To determine which serotypes were circulating in these areas, 26 serum samples were screened by RT-PCR technique. Concurrent infections were observed by three serotypes in two patients, while duel infections were reported in eight patients. Infection of single serotypes was noted in eleven patients. This is the first report documenting concurrent infections by multiple serotypes in patients from India.

**Detection of DEN serotypes by RT-PCR**

| DEN | Number of patients (n=26)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| serotypes | Kerala | Gujarat | Maharashtra | | | | | |
| detected | | | Solapur | Pune | Beed | Kolhapur | Nagpur | Osmanabad |
| 1 + 2 + 4 | - | 2 | - | - | - | - | - | - |
| 1 + 4 | - | 1 | 1 | - | - | - | - | - |
| 1 + 2 | 3 | - | 1 | - | - | - | - | - |
| 2 + 4 | - | - | - | 1 | - | - | - | - |
| 2 + 3 | - | - | - | 1 | - | - | - | - |
| 2 | 2 | - | - | 3 | 1 | 1 | 1 | - |
| 1 | 2 | 1 | - | - | - | - | - | - |

* Viral RNA not detected in 5 cases

## 8. Surveillance of zoonoses acquired from laboratory animals

Experimental output of biomedical research is utilized for human beings. Therefore it is the pre-requisite that the laboratory animals used for such research should be healthy. Animals with a clear microbiological background and a microbiologically controlled rearing environment are indispensable for high precision animal experiments. Healthy and contented animals provide a better experimental. Laboratory animals must be free from all diseases communicable to man and also free from their own pathogens. Therefore microbiological monitoring and testing for disease diagnosis of laboratory animals is essential.

## Objectives

- Monitoring different species of laboratory animals for viral diseases
- Monitoring of laboratory animal staff for zoonotic viral diseases
- Monitoring of laboratory animals & animal handlers for bacterial zoonoses
- Monitoring of laboratory animals & professionals for parasitic, mycotic & rickettsial diseases

## Achievements

Serum samples of various strains of mice (Swiss Albino, NMRI nude, NIH nude, BL/6 nude, BALB/c, DBA/2, C3H/HeJ) collected randomly were tested by ELISA using the diagnostic reagents procured from M/s. Charles River Inc., USA for antibodies against important murine viruses viz. Reo-3, Sendai, Mouse Hepatitis Virus (MHV), Ectromelia and Lymphocytic Choriomeningitis Virus (LCMV). Fortyfive serum samples tested for Reo-3, 36 for Sendai and 37 for LCMV were found negative. Similarly, two serum samples each of Charles Foster, Sprague Dawley & Wistar rats were negative forantibodies to Sendai and LCM viruses. Two serum samples of Hamsters were negative for antibodies to Sendai virus.

IFA assay performed for detection of Reo-3 antibodies, 8/19 mice sera were found positive. 4/8 positive sera were retested and confirmed. For LCM, 1/27 serum sample was positive. Reproducible result was obtained with the positive serum for LCM. These 27 sera include 5 human sera (from NIV animal handlers). For Sendai antibodies all the 15 sera tested were found negative. Similarly, all the 27 sera tested for Ectromelia were found negative.

Twenty-three of the 46 sera from different strains of laboratory mice were found reactive for MHV virus by ELISA. The mouse strains, which were found positive, are C3H/HeJ, C57BL/6 and Golden. All the sera from the mice maintained in isolators were negative indicating the efficacy of using isolators for limiting the spread of infections like MHV, which is transmitted by aerosol route. All the animals used for testing are kept under observation and will be used for confirmation of the results and future studies.

A total of 437 blood samples from animal handlers and control group have been collected from various localities. 133/297 samples from animal handlers were found positive for IgG antibodies against HEV. Whereas, all 221 samples tesetd were negative for anti HEV-IgM antibodies. Screening for antibodies against leptospira by Microscopic Agglutination Test (MAT) revealed that 13/221samples were positive. 3 / 301 samples from animal handlers and 1 / 73 cattle sera were found reactive for brucella antibodies by RBPT.

Stray dogs sera ( n= 45) were tested for leptospira and HAV antibodies. 5 / 45 and 11 / 45 sera were found reactive for leptospira and HAV antibodies respectively.

Sporadic mortality was reported in wild rats. A total of 44 rats of different species, age and sex mostly in moribund stage were collected from the different parts of Pune city. Necropsy of all the rats was performed. Cysts were observed on the livers of 10 / 44 animals. The adult parasites were clearly visible in one case while cutting the affected part of the liver. These were later confirmed to be *Capillaria hepatica* by histopathology. Sera of all the rats were also tested for the presence of leptospira. All the 44 samples from wild rats were negative for antibodies against leptospira by MAT.





## 9. Quality Control Programme

Analysis of pelleted rodent feed samples done at Disease Investigation Section, Pune for presence of Mycotoxins/Aflatoxins. The feed samples found to be free from toxins.

Bacteriological examination of drinking water sample carried out at State Public Health Laboratory, Pune. The water sample found fit for drinking purpose.

Parasitological examination of fecal samples of nude mice maintained in isolator done at Disease Investigation Section, Pune. The samples found to be negative for parasitic eggs or ova.

Post-mortem examination of sick/dead animals done at the Regional Disease Investigation Laboratory, Pune for finding probable cause of death.

## Summary

Seroprevalence of Reo-3 and MHV was noted.

All the sera from animals maintained in Isolators and IVCs were found negative for MHV antibodies indicating the efficacy of these in preventing the spread of infections by aerosol route.

The sera from different categories of animal handlers were collected (n = 437) which constitute important occupational risk group for some of the very important zoonotic agents. Some of the animal species are important in spreading such zoonoses. Considering the importance of seroprevalence studies in animals also we have collected animal sera viz. Stray dogs (n = 45), Cattle (n = 73) and wild rats (n = 44 ). The results of seroprevalence studies indicate 133 / 297, 13 / 221 and 3 / 301 sera reactive for HEV, Leptospira and Brucella respectively in animal handlers. Five out of 45 and 11 / 45 dog sera were found reactive for leptospira and HEV antibodies respectively. All the rat sera were negative for leptospira antibodies. 1 / 73 cattle serum was found reactive for brucella antibodies.

In another study we found parasitic cysts on the livers of wild rats( 10 / 44). The parasite was later confirmed to be *Capillaria hepatica* by gross observations and histopathology of the affected parts of the liver.

## Future plan

Procurement of diagnostic kits, ELISA antigens and control sera required for screening of laboratory animals for murine viruses

Establishment of Individually Ventilated Cage rack systems for housing experimental animals

Screening of the staff of the Division of Lab Animals for laboratory animal allergens

# NATIONAL INSTITUTE OF VIROLOGY

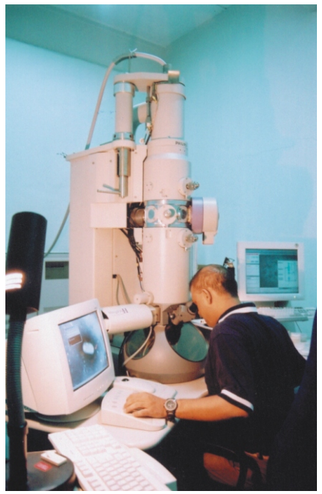
## BUDGET 2003-04

| SR.NO. | BUDGET HEAD | NON PLAN | PLAN | TOTAL |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1 | Pay & Allowances | 572.90 | 207.30 | 780.20 |
| 2 | Pension | 149.50 | 81.75 | 231.25 |
| 3 | Other Charges | 182.85 | 259.79 | 442.64 |
| 4 | Equipment | - | 353.10 | 353.10 |
| 5 | T.A. | 42.95 | 24.61 | 67.56 |
| 6 | Capital | - | 529.00 | 529.00 |
| | TOTAL : | 948.20 | 1,455.55 | 1,874.75 |





# SERVICES AND SUPPLIES



## Electron Microscopy

### Applied ultrastructural research component as a core facility

The Div of EM provided high resolution EM based applications research support to various NIV investigators in relation to virus diagnostics, support to outbreak investigations, ultrastructural cytopathology and routine screening in relation to quality assurance programs. Areas included hepatitis, arboviral investigations and examination of material for rapid virus-morphodiagnosis. Scientists from other non-ICMR research organizations like National Chemical Laboratory and biotechnology R&D cells were also assisted with EM based applications research with appropriate approval.

### Participation in global quality assurance program for virus electron microscopy

Continued participation in the global External Quality Assurance (EQA) program for rapid virus morphodiagnosis conducted by the Robert Koch Institute, Berlin, Germany was carried out and excellent scores achieved for rapid virus detection by EM. The facility is currently in the 17 EQA run.

## Animal House

The following species/strains of animals have been successfully bred and maintained:

Mice Swiss Albino, BALB/c, C3H/HeJ, C57BL/6, BL6 nude, NMRI nude, NIH nude, Golden Colour

Rats Wistar, Sprague Dawley, Charles Foster

Hamsters, Guinea Pigs, Rabbits, Fowls, Geese, Sheep

The number of animals maintained during the year ranged from 1536 to 1465. A total of 3504 animals of different species including 1525 infant groups & 1326 adult Swiss Mice, 599 BALB/c mice were supplied to the scientists of this institute. 1089 surplus animals were supplied on request to various research organizations registered with the CPCSEA and a sum of Rs. 1,27,440/- has been collected towards handling charges and credited to the Generated Funds A/c of A.H.

Blood samples from various species of laboratory animals (Guinea-pigs, Rabbits, Sheep, Fowls/Chicks, Geese) supplied to NIV scientists and outside research institutions on request research purpose.

### Supply of laboratory animals

| Species/ Strains | No. Supplied | | Species/ Strains No. | No. Supplied | |
|---|---|---|---|---|---|
| | NIV | Other institutions | | NIV | Other institutions |
| Mice | | | Rats | | |
| Swiss albino (A) | 1326 | 325 | Wister | 1 | 98 |
| Swiss albino (I) | 1325 | | Sprague Dawly | | 12 |
| BALB/c | 599 | 157 | Charles Foster | | 24 |
| C57BL/6 | 4 | 11 | | | |
| C3H/HeJ | 16 | | Hamster | 2 | |
| Golden | 5 | | Gunea Pigs | 2 | 31 |
| NIH nude | | 194 | Rabbits | 2 | 5 |
| NMRI nude | 8 | 224 | Fowls | 6 | 1089 |
| BL/6 nude | 8 | 8 | | | |
| **Grand Total** | | | | **3504** | **1089** |

### Supply of blood samples

| USER | GUINEA PIG | RABBIT | SHEEP | FOWL | GEESE | TOTAL |
|---|---|---|---|---|---|---|
| NIV SCIENTISTS | 160 ml | -- | 60 ml | 149 ml | 58 ml | 427 ml |
| OUTSIDE | 10 ml | 305 ml | 380 ml | -- | -- | 695 ml |
| TOTAL USE | 170 ml | 305 ml | 440 ml | 149 ml | 58 ml | 1122 ml |
| PERCENT USAGE | 15.15 % | 27.18% | 39.22% | 13.28% | 5.17% | |

## CPCSEA Activities

A Special Meeting of the Institutional Animals Ethics Committee (IAEC) was held on 25th April 2003 for evaluation/approval of the research project entitled *"Transmission and Pathogenesis of Severe Acute Respiratory Syndrome (SARS) virus in experimentally infected rhesus monkeys".* The Committee approved the protocol. The project has been submitted to the Expert Consultant, CPCSEA, Chennai and other authorities for grant of approval.

A Meeting of the Institutional Animals Ethics Committee (IAEC) was conducted at NIV, Pune on 18th July, 2003 for evaluation/approval of 6 research projects submitted by the NIV scientists. The meeting was held under the Chairmanship of Dr. M.G. Deo.

A meeting of the sub-committee comprising Dr. M.G. Deo, Chairman, IAEC, Dr. Vikram Ghole, Member, IAEC and Prof. U.C. Chaturvedi, EMS (CSIR), Lucknow was conducted at NIV, Pune on 27th August, 2003 for evaluation/approval of research project entitled "Comparison of the immunogenic potential, antigenicity, stability of inactivated JEV antigens obtained from Cx. tritaeniorhynchus with that of mouse brain derived antigen" submitted by Dr. Aurobindo Roy.

Research projects on animal experimentation invited from the scientists and forwarded to the Institutional Biosafety Committee and IAEC Members for scrutiny/evaluation. IAEC Meeting is being planned.

Various species/strains of animals were successfully bred, maintained and supplied. A total of 3504 animals of different species were supplied to the scientists of this institute. 1072 surplus animals were supplied outside and a sum of Rs. 1,27,440/- collected.

Research projects on animal experimentation received from the scientists were evaluated/approved by the Institutional Biosafety Committee and Institutional Animal Ethics Committee.

Attempts are constantly being made to meet the various requirements laid down by the IAEC/CPCSEA pertaining to the Laboratory Animals. This involves

Screening of laboratory-bred rodents for important murine viruses by using imported ELISA kits.

Genetic monitoring of inbred strains of mice.

Replacement of indigenous PVC isolators with Individually Ventilated Cage rack system for ease and improved efficiency.

## Virus Registry

Thiteen thousand two hundred and sixty-nine clinical specimens were received from various Government health departments, medical institutions as well as private and general hospitals in different states of India.

### THE BREAK UP OF THE SPECIMENS RECEIVED FOR VIRAL DIAGNOSIS IS AS FOLLOWS.

| Specimen | No.of samples |
|---|---|
| Human | 11,104 |
| Animal | 1,623 |
| Convalescent | 450 |
| Mosquitoes | 91 |
| Virus | 1 |
| Total | 13,269 |

NIV, Pune is a WHO collaborative center for reference and research on Arboviruses and hemorrhagic fever group of viruses. Reference virus strains, virus antigens and immune sera are routinely supplied on request to various research laboratories in India and abroad. The Virus Registry Section has a repository of six hundred virus strains.

## Preparation and supply of virus strains

1) One hundred and sixty five virus strains were supplied to the scientists of various laboratories of India and of NIV.

2) From April 2003 to March 2004, Nine Virus pools of Dengue-2 (P-23085), Dengue-3 (664481), Dengue-4 (611319), Sandfly fever (692643-1), JE (9119645) and West Nile (80235, 82130, 80714, and 80751) were prepared in mouse brain.

3) Mice brains of Thirty-one West Nile isolates, Polio-1, 2, 3 and Echo-25 virus strains were received from Bangalore Field Station.

## Information Centre and Library Services

The NIV Library has procured 110 books, subscribed for 60 journals And received 100 journals on gratis.

### OVID full text database

Full Text Journals database was subscribed and added to NIV-ICL services. Full text services are continued on demand basis. During the year articles were sent for publication through library and all the relevant documentation work was carried out in NIV-ICL. Inter library loan, Xeroxing, Reprint request, Online access, services were continued with addition of some more libraries and biomedical organizations of DST, DBT, CSIR and UGC.

## Maintenance

The services of the maintenance staff was efficiently utilized for satisfactory operation of the equipments installations of the Institute with the available resources and without major downtime.

Jobs of different nature, like preventive maintenance of continuous operating equipment, break down maintenance of cold and freezer rooms, Central Air-conditioning plants, H.T. & L.T. Electrical Panels, internal electrification system, Refrigeration & Air-conditioning equipments, Laboratory equipments, Staff Quarter Maintenance, etc., totaling about 1157 Jobs has been carried out during the year.

## Major jobs carried out / repairs.

- Replacement of Pump House Cable
- Reconditioning of A.C. Plant Compressors of Old building.
- Guest House renovation with A.C. Rooms
- Modification/ alteration has been made in Room No.7 & 6 for New Conference/ Meeting Hall with A.C, P.A. system & LCD Projector.
- Installation of New Equipments
- Repairs in AHU No.6 and replacement of ICTP in AHU No.2
- Monkey Run at Pashan
- Routine maintenance and repairs of the equipments installed at Bangalore Field Unit by deputing the Maintenance Staff.
- Major repairs for Generator 400 KVA





## PARTICIPATION IN IMPORTANT MEETINGS / SEMINARS / SYMPOSIA BY NIV STAFF

**Dr. A.C. Mishra, Officer In-Charge**

1. Meeting at DHS Office regarding SARS at Mumbai on 4th April 2003.
2. A meeting with DG, ICMR for SARS at New Delhi on 2nd May 2003.
3. An extraordinary meeting of the committee for the purpose of Control & Supervision of Experiments on Animals (CPCSEA) in the Committee Room, Min. of Environment & Forests, Paryavaran Bhavan, New Delhi on 9th May 2003.
4. MIT Women's Engg. College Building Centre by His Excellency Dr APJ Abdul Kalam, Hon'ble President of India at MIT Campus, Kothrud, Pune on 28th May 2003.
5. Meeting of the Committee to review the Action Plan / Contingency Plan and the Activities undertaken for prevention and Control of Dengue/DHF at New-Delhi on 30th May 2003.
6. Workshop on Internal & External Quality Assurance of Laboratory Infrastructure in Maharashtra at Addl. Director of HS (FW & MCH), Kutumb Kalyan Bhavan, Pune on 2nd June 2003.
7. Meeting at New Delhi regarding ICMR activities and interest in the JE EIA test and other tests on 6th June 2003.
8. Meeting regarding Plan Paper of Potential SARS Related Research in India at DBT, New Delhi on 7th June 2003.
9. Indo-US Joint Working Group on Prevention and Control of HIV/STD at Washington, USA 16th to 19th June 2003.
10. Regional Consultation on strengtheningof national capacity for prevention and control of SARS at Chennai from 1st to 3rd July 2003.
11. SAC meeting of ICMR virus unit at Kolkatta on 11th July 2003.
12. Meeting under the chairmanship of Secretary (H), Ministry of Health & FW to discuss management of disease outbreak at New Delhi on 23rd July 2003.
13. Meeting on establishment of Centre for Studies in Integrative Studies at University of Pune on 5th August 2003.
14. 22nd Governing Body of NCCS at Pune on 22nd August 2003.
15. SAC meeting of NARI, Pune on 28th and 29th August 2003.
16. 9th Annual Conference of the Maharashtra Chapter of the Indian Association of Medical Microbiologists and Pre-Conference CME at AFMC, Pune on 6th and 7th September 2003.
17. Workshop on Promoting Wider Use & Recognition of Rapid Diagnostic Tests organized by PATH, New Delhi on 10th September 2003.
18. "Current trends in epidemiology of encephalitis: Global - magnitudes & Indian scenario" at Sanjay Gandhi Institute of Med. Sciences, Lucknow 26th and 27th September 2003.
19. Medical Library Association of India (2003) at NIV, Pune from 3rd to 5th December 2003
20. NIV-CMC Virology Symposium at CMC, Vellore on 6th December 2003.
21. Dengue Net Meeting at Kuala Lumpur, Malaysia from 11th to 13th December 2003.
22. Indo-French Workshop at NIV, Pune on 18th and 19 December 2003.
23. Force meeting on Influenza at ICMR Headquarters, New Delhi on 22nd December 2003

24. SAC meeting of CRME, Madurai on 28th and 29th December 2003.
25. Guest lecture at Malabar Institute of Medical Sciences Ltd, Calicut on 3rd January 2004. Indo-US symposium on Infectious diseases Research & Development at Sir Dorabji Tata Centre, IISC Campus, Bangalore from 6th to 10th January 2004.
26. Governing Body of NCCS, Pune pune on 28th January 2004.
27. Meeting to discuss various issues related to bird flu virus at DGHS, New Delhi on 29th January 2004.
28. Meeting of the Directors/Officer-in-Charges of ICMR at Agra on 6th and 7th February 2004.
29. Expert technical meeting for purchase of DNA micro-array system at NICED, Kolkata on 26th February 2004.
30. Fifth Sir Dorabji Tata Symposium on Leishmaniasis organised by Sir Dorabji Tata Centre, Bangalore on 10th and 11th March 2004.
31. NICD-WHO mtg. on "Vector Borne Diseases Surveillance & Control with special reference to DengueNet" at Bangalore on 16th and 17th March 2004.
32. Meeting on Task Force project on "Epidemiology of viral hepatitis in tribal populations of Orissa and Madhya Pradesh and distribution of genetic markers among HBV HCV infected cases / identification of risk factors" at NIV, Pune on 26th March 2004,
33. Meeting regarding project discussion at KEM Hospital, Pune 30th March 2004.

**Dr. (Mrs.) B.L. Rao, Sr. DD**

1. Participated the Indo-French workshop on Dengue on 17th and 18th December 2003.
2. Gave a lecture on Avian Influenza (H5N1) at NIV during a meeting held by ICMR, Poultry Industries on 30th January 2004.
3. Gave a lecture on Influenza study in Pune and Avian influenza: current situation on H5N1 outbreaks in South-East Asia, at the Agarkar Research Institute, Rotary Club Meeting on 1st March 2004.

**Dr. G. Geeverghese, Sr. DD**

1. Attended the 52nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, held at Philadelphia, Pennsylvania, USA from 3rd to 7th December 2003.

**Dr. (Mrs.) S.D. Kelkar, DD**

1. Attended the XIIIth National IAP Conference of Pediatric Gastroenterology, held at Le'Meridian, Pune from 27th to 29th September 2003.
2. Attended the "Dissemination Workshop" on the Operational Research Project for Integrated Disease Surveillance, sponsored by WHO and USAID, jointly organized by B.J. Medical College, Pune & Directorate of Health Services, Govt. of Maharashtra, held at Hotel Aurora Towers on 3rd Oct. 2003.

**Dr. M.M. Gore, DD**

1. Attended the "Roving Seminar on IPR in Biotechnology" arranged by DBT and NCCS at Pune University on 30th Sept 2002.
2. Attended a Workshop on " Biosafety issues related to use of Genetically Modified Organism (GMOs) " held at Hotel Le Meridian, Pune on 20th Sept 2002
3. Participated and presented NIV work on dengue virus at First meeting of Dengue Task force at ICMR Head quarters on 1st October 2002.
4. Was invited speaker faculty during refresher course on trends in Immunology





Workshop on immunology sponsored by Indian Academy of Sciences held at Department of health Sciences University of Pune, Pune.

5. Delivered seminars in College of Military Engineering on "Tackling viral epidemics under NBC readiness."
6. Organization and participant Indo-French Workshop on Tele epidemiology of dengue December 18th and 19th 2003 at NIV.
7. Invited speaker for Indo US Symposium Sir Dorabjee Tata Center, Bangalore from 6th to 10th January 2004. Delivered a talk on "Strategies for vaccine development: Vaccines for travelers *vs.* vaccine for people in endemic region"

**Dr. (Mrs.) V. A. Arankalle, DD**

1. Presented work done at NIV for the ICMR-multi-centric drug trial on hepatitis C on 4th September 2003 at ICMR, Delhi.
2. Delivered a talk entitled " Hepatitis E: Indian Scenario" at CMC, Vellore on 6th December 2003.
3. Participated in a meeting at NIB, Dellhi on 11th December 2003 as a member of the committee set up by DG, ICMR to discuss quality control of imported blood products.
4. Project Review Committee meeting at ICMR on 9th March 2004.
5. Meeting of expert committee on testing of blood products for hepatitis C virus and drug controller of India at NIB, Noida on 15th March 2004.
6. Tribal project meeting at Pune on 26th March 2004.

**Dr. D.T. Mourya, DD**

1. Attended CMC-NIV symposium on Virology, and delivered lead lecture on "Emergence of Dengue & Chikungunya as major public health problem" at Vellore on 5th December 2003.
2. Attended Indo-US symposium, and delivered lecture as panelist on "Role of vector mosquito in the maintenance of dengue viruses in nature" at Bangalore on 5th January 2004.
3. Delivered lead lecture on "Emergence of Chikungunya as major public health problem" on 9th January 2004, XXVII All India Cell Biology Conference & International Symposium organized by Department of Zoology & Institute of Biotechnology & Bioinformatics, University of Pune from 7th to 10th January 2004.
4. Attended as chairperson, symposium on "Recent Trends in Modern Biology", organized by Department of Zoology, 21st March 2004, University of Pune (Sponsored by DBT & DST).

**Dr. A Roy, AD**

1. Attended as an external expert the meeting of Board of Studies of the Department of Life Sciences at Assam University Silchar on 7th April 2003.
2. Attended the 3rd Global Meet on Parasitic Diseases, held at Bangalore University, Bangalore from 12th to 16th January 2004.

**Dr. M.V. Joshi, AD**

1. Attended and participated in the Indo-French Workshop on "Tele-Epidemiology of Dengue viruses" organised by ICMR/CNES held at NIV, Pune on 18th and 19th December 2004.

**Dr. N.S. Wairagkar, AD**

1. WHO Global Training Network Course on Clinical Evaluation of Vaccine Trials at Bangkok Thailand from 3rd to 7th November 2003.
2. WHO workshop on Good Clinical Practices and Good Laboratory Practices training for Vaccine trial, Organized by WHO India, at Chennai from 8th to 10th March, 2004.
3. ICMR epidemiology forum meeting and delivered talk on Encephalitis Outbreak Investigations at NIE, Chennai during October 2003.
4. Indo-French workshop on Tele-epidemiology of Dengue in India on 17-18th December 2003.
5. Annual Conference of Maharashtra chapter of IAMM at AFMC and participated in seminar on Recent outbreak investigations by NIV and delivered lecture on SARS-Current Scenario and Indian perspective during September 2003
6. Indo-US Symposium on Infectious Disease Research and Development and delivered talk on Measles Eradication- Current Scenario, prospects and problems; at Banglore, 6th to 10th January 2004.

**Dr. (Mrs.) M. S. Chadha, AD**

1. Participated as expert in Seminar on Polio-Eradication, Hepatitis A Vaccination under the auspices of School of Health Sciences, Pune University, and CEHAT on 27/09/2003
2. Participated in Brainstorming session: C-DAC & NIV. Topic: GIS & Vector-Borne Disease at C-DAC Pune on 23rd January 2004.
3. Invited for lectures on Epidemiology of Hepatitis B virus and Epidemology and Modes of Transmission of Hepatitis C Virus. at the workshop "Transfusion Medicine Update 2004" held by the Janakalyan Blood Bank Pune, on 25th March 2004

**Dr. (Mrs.) S. D. Chitambar, AD**

Attended 'Biotec India International 2003: International Exhibition and Conference on Biotechnology' at HITEX, Hyderabad (India), on 24-27 Sept 2003 (SD Chitambar).

**Dr. Cecilia D., SRO**

1. ICMR-Zeiss workshop on Confocal Imaging and applications Held at Tuberculosis research centre, Chennai, 7th to 8th April 2003
2. Indo-French workshop organized by ICMR/CNES on Tele-Epidemiology of Dengue from 18th - 19th December 2003 at NIV.
3. Meeting at ICMR Headquarters with IAVI representatives regarding initiation of a trial with the Adeno-associated virus based HIV vaccine on 22/3/04
4. Visiting scientist at Swedish Institute for Infectious Disease Control, Sweden from 6th June to 6th December 2003.

**Dr. C.G. Raut, SRO**

1. VII Zonal Workshop on Laboratory Animal Sciences and Welfare organized by CPCSEA, at National Institute for Research in Reproductive Health (NIRRH), Mumbai, April 21-22, 2003. Delivered a lecture on "Maintenance & Care of Lab Animals".
2. Participated in the Dissemination Workshop on the "Operational Research Project for Integrated Disease Surveillance" sponsored by WHO & USAID jointly organized by B.J. Medical College, Pune & Directorate of Health Services, Govt. of Maharashtra held on 3rd October, 2003 at Pune.
3. Attended the Institutional Animal Ethics Committee Meeting of Raj Udyog, Biotech Division held on 3rd December 2003 at Pune.
4. Participated in the "National Workshop on Biomedical Waste" held at Choithram Hospital & Research Centre, Indore on February 21-22, 2004. Presented a paper entitled "Biomedical waste in lab animal facilities at research institutions". Third Prize awarded for the Poster presented.
5. Invited to attend the Meeting of the Institutional Animals Ethics Committee (IAEC) at S. Nijalingappa Medical College & Hanagal Shri Kumareshwar Hospital & Research Centre, Bagalkot on 1st March 2004 for evaluation/approval of research projects.
6. Participated in the "WHO Sponsored National Workshop on "Fluorescent Antibody Technique for Rabies" held at Department of Neurovirology, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore during March 25-26, 2004.

**Mr. P. N. Yergolkar, SRO.**

1. Participated in the meeting of the Bangalore Mahanagara Palike on review of communicable diseases situation in Bangalore city area - concerned to information on dengue on 27th June 2003.
2. Participated in Polio Lab Co-ordinators's Meeting held at NPSU, New Delhi on 14th July 2003.
3. Participated in 12th Meeting of Virologists from WHO - SEAR Polio Lab Network and 2nd Regional meeting on containment Stocks of Wild Polioviruses from at Colombo, Srilanka from 30th September to 10th October 2003.
4. Participated in Workshop on Quality Assurance in Polio Network Laboratories at E.R.C., Mumbai from 24th to 26th November 2003.
5. Participated in ICMR-WHO Workshop on Intellectual property Rights and World Trade Organisation Issues at R.O.H.C (Southern), Bangalore on 18th and 19th December 2003.
6. Participated in Indo-US Sysmposium on Infectious Deseases Research & Development at Indian Institute of Science, Bangalore from 6th to 10th January 2004.
7. Participated in the 5th Sir Dorabji Tata Symposium on Leishmaniasis at Indian Institute of Science, Bangalore on 10th and 11th March 2004.
8. Participated in NICD-WHO Meeting on Vector Borne Diseases Surveillance and Control with Special Reference to DengueNet at NICD Plague Surveillance Unit, Bangalore on 16th and 17th March 2004.

**Mr. P. George Jacob, SRO.**

1. Participated in Indo-US Sysmposium on Infectious Deseases Research & Development at Indian Institute of Science, Bangalore from 6th to 10th January 2004.
2. Participated in NICD-WHO Meeting on Vector Borne Diseases Surveillance and Control with Special Reference to DengueNet at NICD Plague Surveillance Unit, Bangalore on 16th and 17th March2004.

**Miss. J.P. Thakare, SRO.**

1. Participated in National Review Meeting on Japanese Encephalitis held at NAMP, New Delhi and delivered a lecture on "Laboratory Diagnosis options/relevance" on 20th and 21st June 2003.
2. Attended Biotech India International 2003, International Exhibition and Conference on Biotechnology at Hyderabad from 24th to 27th September 2003.

**Dr. A. Basu**

1. Participated in Indo-French Symposium on GIS systems in infectious diseases at NIV Pune
2. Invited faculty/participant to the 11th Basic Lab Course on EM in infectious Disease, The Robert Koch Institute, Berlin Germany, March 25-26, 2004

**Dr. V. Gopalkrishna, SRO**

1. Delivered a lecture on "oncogenic viruses and Human papilloma virus" related cancers to post graduate summer training students visited at NIV, 17th May 2003.
2. Delivered lecture on Mycoplasma infections in cell cultures to the delegates Training Course on "Basic techniques in Tissue Culture and Virology" conducted by Tissue Culture and Cell Biology Department, 15th May 15th June 2003
3. Invited talk on "DNA viruses and DNA tumour viruses" to M.Sc. Biotechnology students, Pune University, Pune 15th Sept 2003.
4. V. Gopal Krishna, R.S. Tomar, M.G. Pol and A. Jamkar. Co-infections of human papillomaviruses (HPV's) and Epstein Barr virus (EBV) in oral cancers. 23rd Annual convention of the Indian Association for Cancer Research at Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer, Kharghar, Navi Mumbai from 29th to 31st January 2004.
5. Participated in the International Symposium on "Molecular Medicine and Cancer: Contemporary Issues" organized by the department of Molecular Medicine and Biology, Jaslok Hospital on 1st Feb. 2004 at Taj Lands End, Mumbai.

**Mr. S.N. Singh, Library & Information Officer.**

1. Attended a conference, "Trend in Biomedical Librarians" as Rapporteur General at T.M.C., Mumbai, on 12th April 2003.
2. Attended a seminar on "Sun Developer Day", A Sun Technology at La Meridien, Pune on10th December 2003.
3. Attended MLAI meeting on 30/3/03 at New Delhi.
4. Attended a meeting on E-Journals & E-Publishers, Library Partnership organized by M/S Informatics, India at Mumbai on 7th November 2003.

**Mr. J.S. Kalsy, RO**

Attended Medical Library Association of India (MLAI) 2003 Convention held at MCC, Pashan, and presented the articles viz. 1.An Exceptional Approach of an Epidemiologist. 2.Therapeutic effects of Traditional Indian Classical Music 3rd to 5th December 2003.





**Mr. P.S. Shah, RO**

1. ICMR-Zeiss workshop on Confocal Imaging and applications, held at Tuberculosis Research Centre, Chennai from 7th to 8th April 2003.
2. Visited Nagasaki, Japan under RONPAKU fellowship programme awarded by Japan society for promotion of science (JSPS) from 27th September to 26th December 2003.

**Mr. R.S. Jadi, RO**

Attended WHO sponsored 10 days workshop on molecular biology techniques at NICD, Delhi from 3rd to 12th December 2003.

**Mr. S. R. Vaidya**

1. Swadeshi Arogya Mela held at Mumbai and and presented the current work and achievements of NIV in the form of posters from 11th 13th January 2004.
2. BioExpo-2004 held at Vidya Pratishthan's School of Biotechnology, Baramati and presented the current work and achievements of NIV in the form of posters on 3rd and 4th February 2004.
3. Training course "Online Searching of Biomedical databases search" at National Informatics Center (Ministry of Communication & Information Technology, Government of India) New Delhi from 15th to 19th September 2003.
4. 10th FAOB Congress symposium on "Genome Informatics: Problems, Challenges and Perspectives" at Institute of Genomics and Integrative Biology, Delhi held on 5th and 6th December 2003.

**Mrs. V.V. Yewale, Asst. Library & Information Officer.**

1. Demonstration of RFID and Smart Card (LIBSYS Corporation Ltd) at TIFR, Mumbai on 20th June 2003.
2. MLAI-2003, National Convention on "Bridging Traditional Biomedical Knowledge and Modern Science in India and Role of Information Centres and Libraries" held at NIV Pune from 3rd to 5th December, 2003.

**Dr. M. D. Gokhale, TO**

1. Participated in "Swadeshi Arogya Mela" held at Mumbai between 8th to 11th January 2004.
2. Participated in workshop organized by cDAC Pune on "Application of GIS on communicable diseases" held at cDAC Pune on 23rd January 2004.

**Mr. A.M. Walimbe, Junior Biostatistician**

Attended a short course on "Survival Analysis" at Christian Medical College, Vellore from 9th to 13th June 2003.

**Mr. Dominic Fernandes, Sr. Library & Information Asstt.**

1. Demonstration of RFID and Smart Card (LIBSYS Corporation Ltd) at TIFR, Mumbai on 20th June, 2003.
2. MLAI-2003, National Convention on "Bridging Traditional Biomedical Knowledge and Modern Science in India and Role of Information Centres and Libraries" held at NIV Pune from 3rd to 5th December 2003.

**Mr. S.S. Bedekar, STA**

Attended "Vishesh Hindi Karyashala" at Dalhausie from 15th to 17th October 2003

**Dr. (Mrs.) N.J. Shaikh, STA**

1. WHO workshop on Good Clinical Practices and Good Laboratory Practices Training for Vaccine trial, Organized by WHO India, at Chennai, from 8th to 10th March, 2004.
2. XIVIII National congress of Indian Association of Medical Microbiology at Mumbai and presented the paper entitled "Cross neutralization studies on different measles virus strains" from 5th to 9th November 2003.

**Mr. R.M. Kolhapure, STA**

Attended and presented a paper at the International Conference on Disease Management for Sustainable Fisheries (ICON-DMSF, 2003) held from 25th to 28th August 2003 at Trivandrum.

**Mr K. Vijayasimha, RA**

1. Course on "Utilization of IT Tools in Government" Module I conducted by National Informatics Centre at Pune from 10th to 21st November 2003.
2. Course on "Utilization of IT Tools in Government" Module II conducted by National Informatics Centre at Pune from 2nd to 13th February 2004.

**Dr. (Mrs.) V.J. Lad**

Attended "National Seminar on Modern Biology" at Nagarjuna University, Nagarjunanager, Andhrapradesh from 28th 30th August 2003.

**Mr. S.M. Jadhav, RA**

1. Course on "Utilization of IT Tools in Government" Module I conducted by National Informatics Centre at Pune from 9th to 20th June 2003.
2. Course on "Utilization of IT Tools in Government" Module II conducted by National Informatics Centre at Pune from 28th July to 8th August 2003.

**Mr. S.S. Tikute RA**

Participated in Tissue Culture Training Programme (one month) in Tissue Culture Department at NIV.

**Ms. Pragya Yadav, RA**

Attended workshop "Sequence Based Bioinformatics Approach" from 14th to 18th December 2003.

**Mrs. Vandana Chandere, Library & Information Asst.**

1. Demonstration of RFID and Smart Card (LIBSYS Corporation Ltd) at TIFR, Mumbai on 20th June, 2003.
2. Online Searching of Biomedical Databases at NIC, New Delhi from 15th to 19th September 2003.
3. MLAI-2003, National Convention on "Bridging Traditional Biomedical Knowledge and Modern Science in India and Role of Information Centres and Libraries" held at NIV Pune from 3rd to 5th December 2003.





**Mr. S.V. Nipunage, TA**

1. Participated as a Member of the Registration Committee in the MLAI-2003 National Convention of Medical Library Association of India (MLAI), held at NIV, Pune during 3rd to 5th December 2003.
2. Participated in the National Workshop on Biomedical Waste held at Choithram Hospital & Research Centre, Indore. Presented a poster entitled "Biomedical waste in lab animal facilities at research institutions" and was awarded third prize on 21st and 22nd February 2004.
3. Participated in the Hindi Workshop organized by NIV and assisted in organization of Workshop and preparation of Leaflet in Hindi on "Use of Lab Animals" released during this Workshop on 26th and 27th February 2004.

**Dr. D.R. Patil, TA**

Participated in the 4th Indian Veterinary Congress & XI Annual Conference of IAAVR & Symposium on "Newer Concepts and Challenges in Animal Health and Production in the wake of WTO, IPR, IT, Trade Barriers and Certification of Standards" organized at IVRI Izatnagar, UP and presented a paper entitled "Seroprevalence of natural murine viral infections in laboratory mice" on 28th and 29th February 2004.

**Mr. M.P. Rajarshi, Technician**

Participated as a Delegate in the MLAI-2003 National Convention of Medical Library Association of India (MLAI), held at NIV, Pune during 3rd to 5th December 2003.

**Mr.S.N. Fulari, Technician**

Participated in the Hindi Workshop organized by NIV, Pune on 26th and 27th Feb. 2004.

**Miss R. A. Ashtekar, Technician**

Participated in the workshop on Knowledge Discovery in Life Sciences: Sequence based Bioinformatics Approaches. Organized by Bioinformatics Center, University of Pune 14th to 17th December 2003.

**Miss P.R. Patil, JRF**

Attended and presented a paper at the International Conference on Disease Management for Sustainable Fisheries (ICON-DMSF, 2003) held from August 25-28, 2003 at Trivandrum.

**Mr. Rajeev, JRF**

Attended workshop "Sequence Based Bioinformatics Approach" from 14th to 18th December 2003.

**Mr. Sandeep Kumar,**

Attended XXVII All India Cell Biology Conference & International Symposium, Jan 7-10, 2004, organized by Department of Zoology & Institute of Biotechnology & Bioinformatics, University of Pune, Pune 411007, India.

**Mr S M Garbhe, Ex-SAO**

Attended Hindi Conference at Varanasi held from 15th to 17th October, 2004.

**Mrs. A. V. Varkey, A/C Officer**

Attended the workshop on "Cash, A/C's, budgeting for Govt. Departments" held from 24th to 26th April 2003 at Centre for Training and Social Research, New Delhi.

**Mr. P. Subramanian PA**

1. Course on "Utilization of IT Tools in Government" Module I conducted by National Informatics Centre at Pune from 10th to 21st June 2003.
2. Course on "Utilization of IT Tools in Government" Module II conducted by National Informatics Centre at Pune from 2nd to 13th November 2003.

**Mr. B.K. Wadke, PA**

1. Course on "Utilization of IT Tools in Government" Module I conducted by National Informatics Centre at Pune from 9th to 20th June 2003.
2. Course on "Utilization of IT Tools in Government" Module II conducted by National Informatics Centre at Pune from 28th July to 8th August 2003.





## PAPERS PUBLISHED BY NIV SCIENTISTS

1. Arankalle VA, Chadha MS. Who should receive hepatitis A vaccine? (Review) ***J Viral Hep*** 2003; 10: 157-58.
2. Arankalle VA, Murhekar KM, Gandhe SS, Murhekar MV, Ramdasi AY, Padbidri VS, Sehgal SC. Hepatitis B virus: predominance of genotype D in primitive tribes of the Andaman and Nicobar Islands, India (1989-1999). (Short communication). ***J Gen Virol*** 2003; 84: 1915-920.
3. Barde PV, Rane SR, Singh DK, Yadav P, Dighe V, Gokhale MD, Mourya DT.
4. Concomitant effect of *Bacillus thuringiensis* H-14 toxin and Atropine sulphate on the gut epithelial cells of female *Aedes aegypti* mosquitoes. ***Entomon*** 2003; 28(2): 161-63
5. Basu A, Ghosh K. HHV6: Catalyst of genomic lesion in dysfunctional hematopoiesis? ***Indian J Hum Genet*** 2003; 9: 29
6. Chadha MS, Walimbe AM, Chobe LP, Arankalle VA. Comparison of etiology of sporadic acute and fulminate viral hepatitis in hospitalized patients in Pune, India during 1978-81 and 1994-97. ***Indian J Gastroenterol*** 2003; 22: 11-5.
7. Chauhan RM, Deolankar RP. Desi Ghee is the CLA-Ghee: A challenge for Information Centres to compile Information on this traditional neutraceutical. ***In***: Singh SN, Deodhar VG, Rao MM, Kumar S, Shrivastav SA. ***eds.*** Bridging traditional biomedical knowledge and modern science in India and role of information centres and libraries. ***Proc Natl Conv Med Lib Assoc India,*** *3rd-5th December, 2003,* Pune, M/S Shankul Enterprises, 2003; 53-54p. (Book)
8. Gandhe SS, Chadha MS, Arankalle VA. Hepatitis B virus genotypes and serotypes in Western India: Lack of clinical significance. ***J Med Virol*** 2003; 69: 324-30.
9. Gandhe SS, Chadha MS, Walimbe AM, Arankalle VA. Hepatitis B virus: prevalence of precore/core promoter mutants in different clinical categories of Indian patients. ***J Viral Hep*** 2003; 10: 367-82
10. Geevarghese G, Kanojia PC. Impact of urbanization on the prevalence of tick fauna in Pune and its suburbs, Maharashtra State, India. ***Acarologia*** 2003; 43(1): 3-7
11. Geevarghese G, Mavale MS, Ghodke YS, Kode SS, Cicelia D. Veneral transmission of Japanese encephalitis virus in *Culex quinquefaciatus* and West Nile virus in *Culex bitaeniorhynchus*. ***Amer J Trop Med & Hyg*** 2003; 69(3): 446-47
12. Ghosh K, Iyer Y, Basu A. Virological, serological and hematopoietic colony studies and its correlation with the outcome of severe aplastic anemia. ***Hematol J*** 2003; 4: 292-94
13. Jamgaonkar AV, Yergolkar PN, Geevarghese G, Joshi GD, Joshi MV, Mishra AC. Serological evidence for Japanese encephalitis virus and West Nile virus infections in water frequenting and terrestrial wild birds in Kolar district, Karnataka state, India: A retrospective study. ***Acta Virol*** 2003; 47: 185-88
14. Kalsy JS, Deolankar RP. Therapeutic potential of traditional Indian classical music. ***In***: Singh SN, Deodhar VG, Rao MM, Kumar S, Shrivastav SA. ***eds.*** Bridging traditional biomedical knowledge and modern science in India and role of information centres and libraries. ***Proc Natl Conv Med Lib Assoc India,*** *3rd-5th December, 2003,* Pune, M/S Shankul Enterprises, 2003; 52-53p. (Book)
15. Kalsy JS, Ray A, Deolankar RP. An exceptional approach of an epidemiologist. ***In***: Singh SN, Deodhar VG, Rao MM, Kumar S, Shrivastav SA. ***eds.*** Bridging traditional biomedical knowledge and modern science in India and role of information centres and libraries. ***Proc Natl Conv Med Lib Assoc India,*** *3rd-5th December, 2003,* Pune, M/S Shankul Enterprises, 2003; 54-55p. (Book)
16. Kanojia PC. Bionomics of *Culex epidesmus* associated with Japanese encephalitis virus in India. ***J Amer Mosquito Control Assn*** 2003; 19(2): 151-54

17. Kanojia PC, Shetty PS, Geevarghese G. A long term study on vector abundance & seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, Uttar Pradesh. ***Indian J Med Res*** 2003; 117: 104-10

18. Kelkar SD, Bhide VS, Ranshing SS, Bedekar SS. Rapid ELISA for the diagnosis of rotavirus. ***Indian J Med Res*** 2004; 119: 60-65

19. Lole KS, Jha JA, Shrotri SP, Tandon BN, Mohan Prasad VG, Arankalle VA. Comparison of Hepatitis C virus Genotyping by 5'noncoding region and core-based reverse transcriptase PCR assay with sequencing and use of the assay for determining subtype distribution in India. ***J Clin Microbiology*** 2003; 41(11): 5240-244.

20. Mourya DT, Singh DK, Yadav P, Gokhale MD, Barde PV, Narayan NB, Thakare JP, Mishra AC, Shouche YS. Role of gregarine parasite *Ascogregarina culicis* (Apicomplexa: Lecudinidae) in the maintenance of Chikungunya virus in vector mosquito. ***J Eukaryot Microbiol*** 2003; 50(5): 379-82

21. Mourya DT, Pidiyar V, Patole M, Gokhale MD, Shouche Y. Mid gut bacterial flora of *Aedes aegypti* affects the susceptibility of mosquitoes to dengue viruses. ***WHO Dengue Bull 2003***; 26: 190-194.

22. Paramsivan R, Mishra AC, Mourya DT. West Nile virus: The Indian scenario***. Indian J Med Res 2003***; 118: 101-08.

23. Paranjape S. Goat serum: An alternative to fetal bovine serum in biomedical research. ***Indian J Exptl Biol*** 2004; 42: 26-35.

24. Paranjape S, Patil BR, Kadam VD. Characterization of porcine stable kidney cell-line adapted to hyperthermic temperature. ***In vitro Cell Dev Biol*** (Animal) 2003; 39: 193-95

25. Paranjape SP, Wagh UV. Virus induced chromosomal abnormalities in Chinese hamster lung cell line and human peripheral blood leukocyte culture. ***Indian J Exptl Biol*** 2003; 41(2): 112-17.

26. Rajarshi MP, Deolankar RP. Experimental zoopharmacognosy and biodiversity gardens. ***In***: Singh SN, Deodhar VG, Rao MM, Kumar S, Shrivastav SA. ***eds.*** Bridging traditional biomedical knowledge and modern science in India and role of information centres and libraries. ***Proc Natl Conv Med Lib Assoc India,*** *3rd-5th December, 2003,* Pune, M/S Shankul Enterprises, 2003; 64-65p. (Book)

27. Ranshing SS, Kelkar SD. Isolation and characterization of dually reactive strains of Group A Rotavirus from hospitalized children. ***J Clin Microbiol*** 2003; 41(11): 5267-69

28. Rao BL. Epidemiology and control of influenza (Review Article). ***Natl Med J India*** 2003; 16(3): 143-49

29. Raut CG, Gengaje BB, Nipunage SV, Rajarshi MP, Vaidya SR, Rane SR, Pol SS, Kohale KN. *Capillaria hepatica* infection in a bandicoot rat (*Bandicota indica*). ***J Vet Parasitol*** 2003; 17(1): 71-2

30. Raut CG, Thakare JP, Padbidri VS, Sapkal GN, Mishra AC, Paramasivan R, Gokhale MD, Mourya DT, Shouche YS, Jayakumar PC. A Focal Outbreak of Japanese encephalitis among horses in Pune District, India. ***J Commun Dis*** 2003; 35(1): 40-42

31. Ray PG, Kelkar SD. Prevalence of neutralizing antibodies against different Rotavirus serotypes in children with severe rotavirus-induced diarrhea and their mothers. ***Clin & Diagnostic Lab Immunol*** 2004; 11(1): 186-94

32. Ray PG, Kelkar SD. Measurement of anti rotavirus IgM/IgA/IgG responses in the serum samples of Indian children following rotavirus diarrhoea and their mothers. ***J Med Virol*** 2004; 72: 416-23

33. Singh SN. ***ed*** Horizon of Information Technology, New Age, New Ways Trend and Impact of on Information and Library Science; Festschrift Vol: I, Pune, Imamdar Bandhu Prakahan, 2003: vi; 288 p (Book).





34. Singh SN. Information Technology (IT) Trends and Prospects ***In****:* Singh SN ***ed*** Horizon of Information Technology, New Age, New Ways Trend and Impact of on Information and Library Science; Festschrift Vol: I, Pune, Imamdar Bandhu Prakahan, 2003: 1-49.

35. Singh SN, Kumar RP, Deodhar VG, Rao MM, Kumar S, Shrivastav SA. ***eds*** `Bridging Traditional Biomedical knowledge and modern science in India and role of Information Centres and Libraries. ***Proc Natl Conv Med Lib Assoc India,*** *3rd-5th December, 2003,* Pune, M/S Shankul Enterprises, 2003; 276p (Book)

36. Singh SN, Garg BS. Bridging traditional biomedical knowledge and modern science in India: an overview. ***In***: Singh SN, Deodhar VG, Rao MM, Kumar S, Shrivastav SA. ***eds.*** Bridging traditional biomedical knowledge and modern science in India and role of information centres and libraries. ***Proc Natl Conv Med Lib Assoc India,*** *3rd-5th December, 2003,* Pune, M/S Shankul Enterprises, 2003; 12-24p. (Book)

37. Singh SN. Patent Information its Needs in Biomedical Research and Development***. MLAI Bulletin*** 2003; 6(2): 30-36.

38. Singh SN. Impact of CD-ROM Technology on Users of Biomedical Information Services in India; a Critical Evaluation***. MLAI Bulletin*** 2003: 6(2): 37-45.

39. Singh S.N, Shrivastav SA. Digitisation of Biomedical Information Centres and Libraries in India: a proposal. ***MLAI Bulletin*** 2003; 6 (2): 75-88.

40. Singh SN. Patent Information; its Need in Biomedical Research and Development***. MLAI Bulletin*** 2003; 6 (2): 89-99.

41. Singh SN. Digitisation: Preservation and Conservation of Indigenous Biomedical Heritage Knowledge in Developing Countries; with special reference to India***. IFLA Int. Conf. (Interlending and Document Supply)*** *Canberra (Australia) 28-31 October,* 2003

42. Vaidya SR, Tilekar BN, Walimbe AM, Arankalle VA. Increased risk of Hepatitis E in sewage workers from India. ***J Occupational & Environmental Med*** 2003; 45(10): 1167-170

43. Venkateswarlu CH, Zade JK, Verma SP, Deolankar RP. Three-dimensional modelling of NSP4 and Enkephalins: A basis for structure based vaccine and drug design for pediatric rotavirus diarrhoes. ***Bioinformatics India*** 2004; 2(1): 19-22.

44. Wadke BK, Deolankar RP. Western ranges (Ghat) Health, Sports and Adventure Tourism: A strategy for treating modern diseases with natural methods. ***In***:

45. Yadav P, Gokhale MD, Barde PV, Singh DK, Mishra AC, Mourya DT. Experimental transmission of Chikungunya virus by Anopheles stephensi mosquitoes. Acta Virol 2003; 47: 45-7

46. Yadav P, Shouche YS, Mishra AC, Mourya DT. Genotyping of Chikungunya virus isolated in India during 1963-2000 by reverse transcription polymerase chain reaction. ***Acta Virol*** 2003; 47: 53-6.

47. Yeolekar LR, Kulkarni PB, Pawar SD, Rao BL. Re-emergence of B/Victoria/2/87 like Influenza virus strain in Pune. ***Cur Sci*** 2004; 86(7): 966-68.

## TRIANING / WORKSHOP / SEMINAR /CONFERENCE ORGANIZED

1. National Convention, entitled," Bridging Traditional Biomedical Knowledge and Modern Science in India and Role of Information Centers and Libraries" was organized during 3-5 December, 2003.
2. Workshop on Diagnosis of Japanese encephalitis was organized from10th to 14th March 2003.
3. Hindi Workshop organized on 26-27 February, 2004 as a part of the Annual Programme of the Department of Official Language, Government of India. Leaflet in Hindi language on "Use of Lab Animals" prepared and released during this Workshop.
4. Trainees of Summer Vacation Course trained in various aspects of laboratory animal science.
5. Two Scientific staff from National AIDS Research Institute, Pune trained in various aspects of Laboratory Animal Science such as care & management and experimental techniques of Laboratory Animals with particular reference to Mice.
6. Scientist from Lab Animal Division of CDRI, Lucknow trained in "Virus isolation and its assay techniques".
7. Student of MD Ayurveda of Government Ayurveda College, Nanded trained in various aspects on Care, Management & Experimental Techniques of Laboratory Animals
8. Lecturer in Pharmacology of Seth Govind Raghunath Sable College of Pharmacy, Saswad trained in various aspects on Care, Management & Experimental Techniques of Laboratory Animals
9. Five days training (15-19 March 2004) imparted to Ms. Jyoti Tope, Ph.D. student Dept. Zoology, The Institute of Science, Mumbai for conducting experiments on Spodoptera litura cell line.
10. Training programme on *Phlebotomine* sandfly biosystematics, from 9-12 August 2003, held at Hyderabad, for medical officers, Govt. of Andhra Pradesh.
11. Biosystematics of sandflies in connection with Chandipura encephalitis; A training workshop of the AP State entomologists, 8-13 September 2003, held at MCC, NIV. Pune





## TRAINING UNDERTAKEN DURING 2003-2004

Dr. D. T. Mourya, Deputy Director, Mr. Pradip V. Barde, Research Assistant & Ms. Pragya Yadav, Research Assistant, acquired 15 days training at High Security Animal Disease Laboratory, Bhopal, to acquire knowledge of working in high containment laboratory.

Dr. D. T. Mourya and Mrs. Veena Vipat attended three days customization training for working on ABI 3100 DNA Sequencer, at Delhi from 9th to 11th March 2004.

## AWARDS / NOMINATIONS

Dr. SN Singh, Library and Information Officer, received the MLAI GM Kumar award for the year 2002, which was conferred during MLAI 2003. National Convention held at National Institute of Virology IICMR) Pune, (Photograph)

Third Prize awarded to the poster entitled "Biomedical waste in lab animal facilities at research institutions" presented by Dr. C.G. Raut and S.V. Nipunage at the National Workshop on Biomedical Waste held at Choithram Hospital & Research Centre, Indore on February 21-22, 2004.

## List of Officers

| NAME | DESIGNAITON |
|---|---|
| DR AC MISHRA | OFFICER IN CHARGE/DY. DIR. (SR.GR.) |
| DR (MRS) BL RAO | DEPUTY DIRECTOR (SR.GR) |
| DR G. GEEVARGHESE | DEPUTY DIRECTOR (SR. GR) |
| DR PVM MAHADEV | DEPUTY DIRECTOR |
| DR MM GORE | DEPUTY DIRECTOR |
| DR (MRS) VA ARANKALLE | DEPUTY DIRECTOR |
| DR DT MOURYA | DEPUTY DIRECTOR |
| DR A ROY | ASSISTANT DIRECTOR |
| DR MV JOSHI | ASSISTANT DIRECTOR |
| DR NS WAIRAGKAR | ASSISTANT DIRECTOR |
| DR RP DEOLANKAR | ASSISTANT DIRECTOR |
| DR (MRS) MS CHADHA | ASSISTANT DIRECTOR |
| DR (MRS) SD CHITAMBER | ASSISTANT DIRECTOR |
| DR PHOOL CHAND | ASSISTANT DIRECTOR |
| MISS SP VERMA | ASSISTANT DIRECTOR |
| DR (MISS) C DAYARAJ | ASSISTANT DIRECTOR |
| DR SN RANADIVE | SR. RESEARCH OFFICER |
| MR PG JACOB | SR. RESEARCH OFFICER |
| MRS SP PARANJAPE | SR. RESEARCH OFFICER |
| DR CG RAUT | SR. RESEARCH OFFICER |
| MR PS SATHE | SR. RESEARCH OFFICER |
| DR (MISS) JP THAKARE | SR. RESEARCH OFFICER |
| MR PRASANNA YERGULKAR | SR. RESEARCH OFFICER |
| DR ATANU BASU | SR. RESEARCH OFFICER |
| DR V GOPALKRISHNA | SR. RESEARCH OFFICER |
| DR (MRS) LR YEOLEKAR | SR. RESEARCH OFFICER |
| MR JS KALSY | RESEARCH OFFICER |
| MR PS SHAH | RESEARCH OFFICER |
| DR (MRS) KS LOLE | RESEARCH OFFICER |
| MR. RS JADI | RESEARCH OFFICER |
| MR SR VAIDYA | RESEARCH OFFICER |
| DR VP BONDRE | RESEARCH OFFICER |
| MR MG POL | SR. TECHNICAL OFFICER |
| MRS SS ATHAWALE | SR. TECHNICAL OFFICER |
| MR SS KADAM | SR. TECHNICAL OFFICER |
| DR SN SINGH | LIBRARY & INFORMATION OFFICER |
| MR ST PERUMAL | MAINTENANCE OFFICER |
| MR YD SABLE | ASTT. MAINTENANCE OFFICER (SR) |
| MR SM GARBE | SR. ADMN. OFFICER |
| MRS. AV VARKEY | ACCOUNTS OFFICER |
| MR VC KHUNYAKARI | SENIOR STORES OFFICER |
| MRS VV YEWALE | ASSTT LIB & INFORMATION OFFICER |
| MR AM WALIMBE | JR BIO STATSTICIAN |
| MR VM PUNEKAR | TECHNICAL OFFICER |
| MR SB DIXIT | TECHNICAL OFFICER |
| MR AV JAMGAONKAR | TECHNICAL OFFICER |
| MR BB GENGAJE | TECHNICAL OFFICER |
| MR DS RAGHAVENDRA RAO | TECHNICAL OFFICER |
| MR MS PAWAR | TECHNICAL OFFICER |
| MR MURALI KRISHNA | TECHNICAL OFFICER |
| MR LV HUNGUND | TECHNICAL OFFICER |
| DR MD GOKHALE | TECHNICAL OFFICER |
| MR HANUMAIAH | TECHNICAL OFFICER |
| MR MR KHUDE | TECHNICAL OFFICER |





| | |
|---|---|
| MR CB LANDE | TECHNICAL OFFICER |
| MR HM SHINDE | SR.TECHNICAL ASSISTANT |
| MR SS BEDEKAR | SR.TECHNICAL ASSISTANT |
| MR JA RATHOD | SR.TECHNICAL ASSISTANT |
| MRS VP SALUNKE | SR.TECHNICAL ASSISTANT |
| MR CD TUPE | SR.TECHNICAL ASSISTANT |
| MR BD WALHEKAR | SR.TECHNICAL ASSISTANT |
| MR GN MULIK | SR.TECHNICAL ASSISTANT |
| MR BD SASTE | SR.TECHNICAL ASSISTANT |
| MR SF NAIK | SR.TECHNICAL ASSISTANT |
| MR K MOHANBABU | SR.TECHNICAL ASSISTANT |
| MR A SINGH | SR.TECHNICAL ASSISTANT |
| DR(MRS) NJ SHAIKH | SR.TECHNICAL ASSISTANT |
| MR AR SABLE | SR.TECHNICAL ASSISTANT |
| MR DL GAIKWAD | SR.TECHNICAL ASSISTANT |
| MR RM KOLHAPURE | SR.TECHNICAL ASSISTANT |
| MR R SHANMUGAM | SR. TECHNICAL ASSISTANT |
| MR MS JAGTAP | SR. TECHNICAL ASSISTANT |
| MRS LP CHOBE | RESEARCH ASSISTANT |
| MRS VJ LAD | RESEARCH ASSISTANT |
| MRS MS MAVALE | RESEARCH ASSISTANT |
| MR VIJAY SIMHA | RESEARCH ASSISTANT |
| DR PV BARDE | RESEARCH ASSISTANT |
| MRS SV GANGODKAR | RESEARCH ASSISTANT |
| DR AB SUDEEP | RESEARCH ASSISTANT |
| MRS RG DAMLE | RESEARCH ASSISTANT |
| MRS PG RAY | RESEARCH ASSISTANT |
| DR(MRS) VA POTDAR | RESEARCH ASSISTANT |
| MR SS TIKUTE | RESEARCH ASSISTANT |
| MR SM JADHAV | RESEARCH ASSISTANT |
| MISS PD YADAV | RESEARCH ASSISTANT |
| MR D FERNANDES | SR.LIB. & INF.ASSISTANT |
| MISS MB THAKKER | LIB & INFORMATION ASSISTANT |
| MRS VS CHANDERE | LIB & INFORMATION ASSISTANT |
| MR AB KHARE | ASSTT MAINTENANCE OFFICER |
| MR RB VANARASE | SECTION OFFICER |
| MR VB DESHPANDE | SECTION OFFICER |
| MR DG THATTE | SECTION OFFICER |
| MR VV PETHE | SECTION OFFICER |
| MR SR ALLE | SECTION OFFICER |
| MR VR JADHAV | SECTION OFFICER |
| MR SL KALEKAR | SECTION OFFICER |
| MR SB ATTAR | PRIVATE SECRETARY |
| MR T SANKARDASAN | PRIVATE SECRETARY |
| MR SS GOLE | ASSISTANT |
| MR UN SHETTY | ASSISTANT |
| MR SS KALE | ASSISTANT |
| MR SB PANDIT | ASSISTANT |
| MRS BA HORNE | ASSISTANT |
| MISS SN PONKSHE | ASSISTANT |
| MR N SHIVSEKHAR | ASSISTANT |
| MR YK MAZIRE | ASSISTANT |
| MR NGS NAIR | ASSISTANT |
| MR AS GAIKWAD | ASSISTANT |
| MR JS RANGAN | ASSISTANT |
| MR VP PANDHARKAR | ASSISTANT |
| MRS SD BHALERAO | ASSISTANT |
| MR DV KULKARNI | ASSISTANT |
| MRS PK RATNAPARKHI | ASSISTANT |
| MR. DT SALUNKE | ASSISTANT |
| MRS AS DESGPANDE | ASSISTANT |
| MRS AS PALSHIKAR | ASSISTANT |
| MR P SUBRAMANIAN | PERSONAL ASSISTANT |
| MR BK WADKE | PERSONAL ASSISTANT |
| MRS RK AMALE | PERSONAL ASSISTANT |

## Institutional Animals Ethics Committee (IAEC)

1. Dr. M. G. Deo, C-13, Kubera Gulshan Apartments, DP Road, Aundh, PUNE 411007
2. Dr. U.V. Wagh, Director, Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth Medical College Campus, Pune-Satara Road, Katra-Dhankawadi, PUNE - 411043
3. Dr.Vikram S. Ghole, Head, Department of Environmental Sciences, Pune University Campus, Ganeshkhind, PUNE 411007
4. Dr. G.R. Ghalsasi, Advisor, Poultry Diagnostic & Research Centre, Venkateshwara Hatcheries Ltd., 18, K.M. Pune-Solapur Road, LONI-KALBHOR 412 201, Haveli, Dist. PUNE
5. Dr. B. Ramanmurthy, Scientist C & Animal House Incharge, National Centre for Cell Science, Pune University Campus, Ganeshkhind, PUNE 411007
6. Ms. Sunanda Das, Blue Cross Society of Pune, 24/1, Koregaon Park, PUNE 411001
7. Dr U. Sengupta, Emeritus Medical Scientist, Central JALMA Instt. for Leprosy, Tajganj, AGRA 282 001
8. Mr. Neelimkumar Khaire, Director, Indian Herpetological Society, Pune, C/o.Rajiv Gandhi Zoological Park & Research Centre, Katraj, PUNE - 411046
9. Dr. A.C. Mishra, Officer-in-Charge, National Institute of Virology, PUNE 411001.
10. Dr C.G. Raut, Senior Research Officer, National Institute of Virology, PUNE 411001.

## Ethical Committee (Humans)

1. Dr V.S. Padbidri, Director, KEM Hospital, Rasta Peth, PUNE 411 011.
2. Dr S.M. Mehendale, Deputy Director, Naational AIDS Research Institute, Plot No.73, 'G' Block, MIDC Bhosari, PUNE 411 026
3. Dr A.S. Bhave, Associate Professor & Unit Head, Medicine Department, BJ Medical College, PUNE 411 001.
4. Mr R.V. Nangare, Advocate, “Yash-Villa”, Near Chinchechi Talim, Off Bajirao Road, PUNE 411 002.
5. Mrs Sunanda Das, Founder Secretary, Red Cross Society of India, 24/1, Koregaon Park, PUNE 411 001.
6. Prof. R.K. Mutatkar, 64, Anand Park, Aundh, PUNE 411 007.
7. Dr R.S. Soman, 43/9-A, Gumpha, Erandawane, PUNE 411 004.
8 Dr A.C. Mishra, Officer-in-Charge, NIV, PUNE 411 001.

## Institutional Bio-safety Committee (IBSC)

1. Dr U.V. Wagh, Director, IRSHA, Pune / DBT Nominee
2. Dr A.C. Mishra, Officer-in-Charge, NIV, Pune
3. Dr Bhaskar Saha, Scientist 'E', National Centre for Cell Science, Pune
4. Dr D.T. Mourya, Deputy Director, NIV, Pune
5. Dr M.S. Chadha, Assistant Director, NIV, Pune
6. Dr Cecilia Dayaraj, Senior Research Officer, NIV, Pune





## Scientific Advisory Committee Members

1. Lt. Gen. D. Raghunath, Principal Executive, Sir Dorabji Tata Centre for Research in Tropical Diseases, Innovation Centre, Indian Institute of Science Campus, BANGALORE 560 012
2. Dr G.C. Mishra, Director, National Centre for Cell Sciences, NCCS Complex, Ganeshkhind, PUNE 411 007
3. Dr T. Jacob John, 439, Civil Supplies, Godown Lane, Kamalakshmi Puram, VELLORE 632 002
4. Dr K.K. Dutta, B-51, Akash Ganga Apartments, Plot No.17, Sect. 6 Dwarka, NEW DELHI 110 045
5. Dr V. Ravi, Additional ProfessoR, Department of Neurovirology, National Institute of Mental Health & Neuro Sciences, BANGALORE 560 002
6. Dr Shobha Broor, Professor, Department of Microbiology, All India Institute of Medical Sciences, NEW DELHI 110 029.
7. Dr M.K.K. Pillai, 37, Sakshar Apartment, A-3, Paschim Vihar, NEW DELHI 110 063.
8. Dr Saheed Jameel, Group Leader (Virology), International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, NEW DELHI 110 067
9. Dr U.C. Chaturvedi, 201, Annapurna Apartments, No.1, Bishop Rocky Street, Faizabad Road, LUCKNOW 226 007
10. Prof. S.C. Lakhotia, Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, VARANASI 221 005
11. Dr S. Basu, Director, National Institute of Immunology, Aruna Asaf Ali Marg, NEW DELHI 110 067
12. Dr A.S. Kolaskar, Vice-Chancellor, University of Pune, Ganeshkhind Road, PUNE 411007.
13. Dr Subhash R. Salunke, Director GeneraL, Directorate General of Health Services, Government of Maharashtra, Govt. Dental College Building, 4th Floor, St. George's Hospital Compound, P.D'Mello Road, Fort, MUMBAI 411 001.
14. Dr Mridula Phadke, Director, Directorate of Medical Education & Research, Government of Maharashtra, Govt. Dental College Building, 4th Floor, St. George's Hospital Compound, P.D'Mello Road, Fort, MUMBAI 411 001.
15. Dr D.A. Gadkari, Emeritus Medical Scientist (ICMR), National Institute of Virology, 20-A, Dr Ambedkar Road, PUNE 411 001.
16. Dr Ira Ray, Additional Director General, Directorate General of Health Services, Nirman Bhavan, NEW DELHI 110 011
17. Dr Lalit Kant, Sr Deputy Director General, Indian Council of Medical Research, Ansari Nagar, NEW DELHI 110 029
18. Dr Dipali Mukherjee, Sr Deputy Director General, Indian Council of Medical Research, Ansari Nagar, NEW DELHI 110 029
19. Dr A.C. Mishra, Officer-in-Charge, National Institute of Virology, 20-A, Dr Ambedkar Road, PUNE 411 001.