# Viral Hepatitis

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# 2 Viral Hepatitis

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

## Development of candidate vaccine for hepatitis E

VA Arankalle, KS Lole, TM Deshmukh.

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

### Objectives

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

### Work Done

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

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

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

### Future plans

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

## Development of combined vaccines for Hepatitis B and E viruses.

Shubham Srivastava, KS Lole, AS Tripathy, VA Arankalle

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

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

## Work done

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

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

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

## Antibody isotype analysis

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

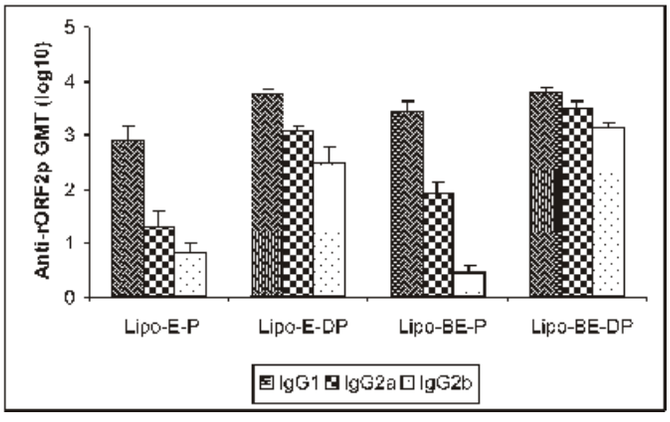



**Fig.1 : Anti-HEV (ORF2) IgG subtype analysis in mice immunized with different liposome formulations**

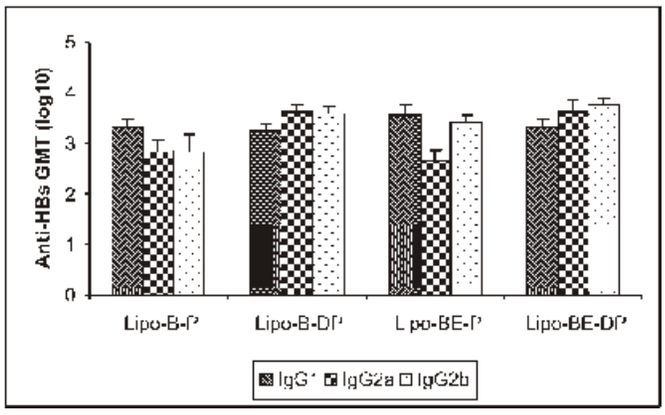



**Fig. 2 : Anti-HBs IgG subtype analysis in mice immunized with different liposome formulations**

### Future plans

Promising vaccine candidates will be evaluated in rhesus monkeys.

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

KS Lole, VA Arankalle

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

### Work done

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

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

### Future plans

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

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

Nischay Mishra, VA Arankalle

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

### Objectives

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

### Work done

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

### Future plans

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

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

Yogesh Karpe, K S Lole

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

### Objective

- To study the processing of ORF1 protein.

### Work done

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

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

### Future plans

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

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

VA Arankalle, MA Kulkarni

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

### Objective

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

### Work done

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

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

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

Vikram Verma, VA Arankalle

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

### Objectives

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

### Work done

### Standardization of multiplex PCR

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

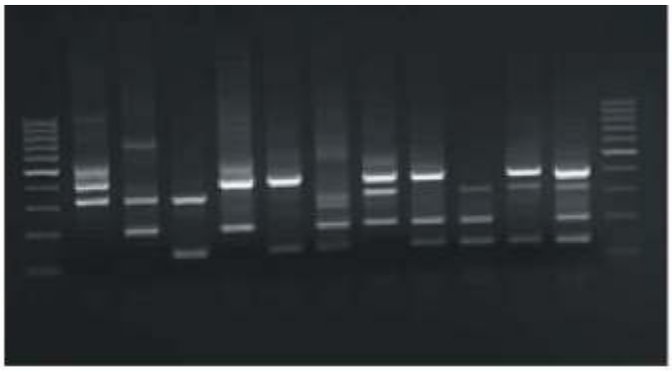



**Fig. 3: Standardization of multiplex PCR**

### Collection of drinking water samples

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

### Collection of river water samples

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

### Testing of the samples

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

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

**Table-2 : Viral RNA positivity by PCR**

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

## Detection of Hepatitis A and E viruses in soil samples

Deepti Parashar, VA Arankalle

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

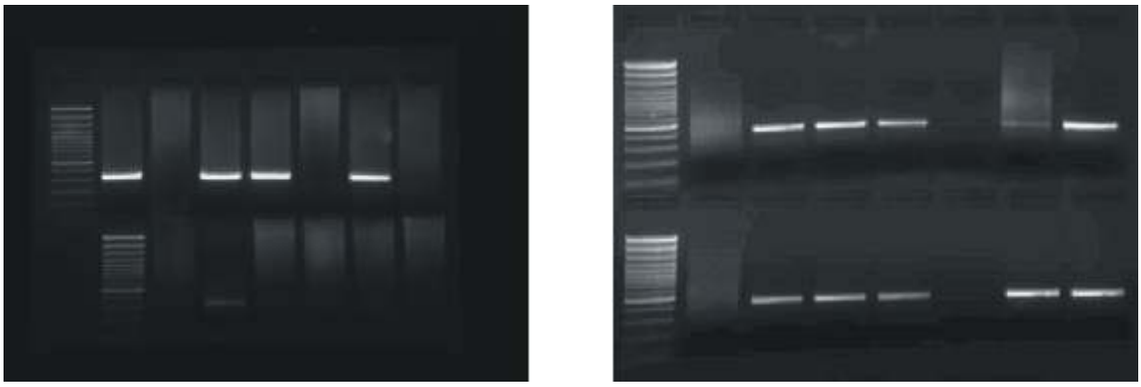
### Objectives

- Detection of Hepatitis A and E virus in soil samples

### Work done

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

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



(a) (b)

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

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

VA Arankalle, MA Kulkarni, LP Chobe

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

### Objectives

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

### Work done

### Molecular surveillance

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

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

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

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

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

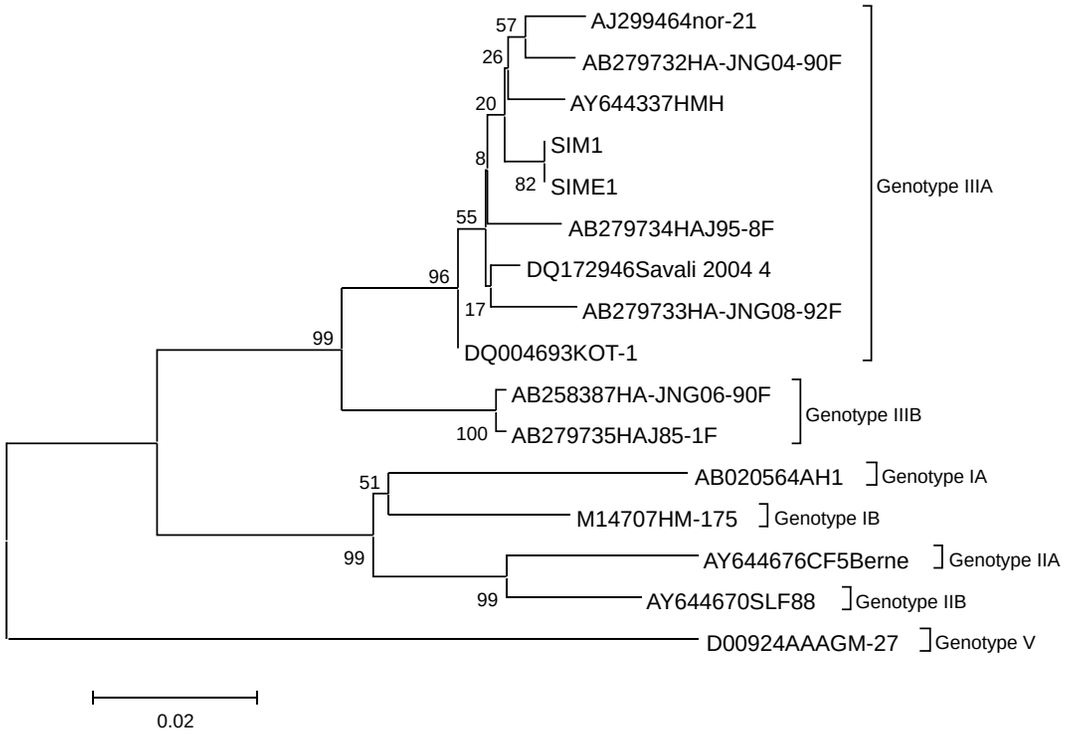



**Fig. 5. Phylogenetic tree based on 5'non-coding region**

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

SD Chitambar, MS Joshi, Shilpa Bhalla

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

### Objective

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

### Work done

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

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

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

VA Arankalle

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

### Work done

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

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

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

Gauri Gupte & VA Arankalle

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

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

### Objective

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

### Work done

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

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

AS Tripathy, VA Arankalle

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

### Objective

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

### Work done

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

### Future plans

Studies will be continued by increasing the sample size.

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

VA Arankalle

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

## Objective

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

## Work done

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

## Services Provided

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

## Publications


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

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

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

## Workshops / Conferences / Seminar / Meetings attended

**Dr. VA Arankalle;**

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

**Dr. KS Lole**

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

**Dr. AS Tripathy**

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

**Dr. LP Chobe**

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

**Dr.TM Deshmukh**

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

**Shubham Srivastava**

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

**Subhashis Chatterjee**

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

## Training programmes / Workshops/ Seminars organized

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