Review of Literature
2. REVIEW OF LITERATURE

HEPATITIS A VIRUS

Hepatitis A Virus (HAV) which has recently been classified as a prototype virus of the new genus-Hepatovirus belonging to the family Picornaviridae is a non-enveloped, spherical particle with a diameter of about 27 nm. The virion consists of a genome of linear single-stranded RNA and a protein shell made up of 3 major proteins VP1, VP2, and VP3. The presence of a fourth protein VP4 has been repeatedly described but conclusive physical identification of the protein is still missing.

The disease due to hepatitis A, often termed 'Infectious Hepatitis' is frequently sub-clinical or unrecognised illness especially in children. In overt disease, the clinical picture ranges from a mild anicteric illness (infection without jaundice) to acute disease which sometimes leads to fatal fulminant hepatitis (< 0.2%) with sudden onset and death. Occasionally it is also a cause of subacute hepatic failure. A notable feature of HAV is that it does not persist in the host, nor is there evidence of progression to chronic liver damage.8

The incubation period of the virus is 2 - 6 weeks during which it is believed to multiply in the gut epithelium. It is then carried by blood to liver, the virus multiplies in the cytoplasm of liver parenchymal cells. The clinical spectrum has 4 phases: The incubation period, Prodromal phase, Icteric phase and Convalescent phase.
The major mode of transmission is by faecal contamination. Virus is excreted in the faeces, mainly during the late incubation period and the first week of clinical illness, but viral shedding in faeces can recur during occasional relapses of acute hepatitis A.\(^9\)

The period of viraemia is short (7 - 10 days) and viral particles are present in blood in relatively low concentrations.\(^{10-14}\)

The specific diagnostic tests include:

i) Detection of HAV particles or components of the virus by Immune electron microscopy (IEM) and Radioimmuno assay (RIA) or Enzyme linked immuno sorbent assay (ELISA).

ii) Detection of a specific antibody response by a variety of serological tests, including haemagglutination, immunofluorescence and ELISA.

The most extensively followed method of detection is the detection of IgM antibody in serum by ELISA which indicates recent infection with HAV. Patients remain positive for IgM for 3 - 6 months after the onset of symptoms. IgG antibody in serum indicates past-infection with HAV and it persists for years conferring long-lasting immunity.\(^8\)

Seven genotypes of the virus have been identified but all of these are encompassed within one serotype and it is assumed that infection with one will provide protection against all.\(^{15,16}\)
Hepatitis A virus is the only one of the six viruses to have been repeatedly isolated and serially passaged in cell culture, albeit with considerable difficulty and this has made it possible to develop a practical hepatitis A vaccine.\textsuperscript{17}

A number of inactivated and live attenuated HAV vaccines have been developed.\textsuperscript{18-20} These include vaccines produced using HM 175 strain (SKB vaccine), H2 strain (China) and KRM 003 strain (Japan.). The SKB vaccine or Havrix developed by the Smithkline Beecham Biologicals, Belgium is commercially available and includes a primary immunisation schedule consisting of 2 doses given 2 weeks to 1 month apart and a booster given 6 - 12 months after the primary course. Antibodies persist for at least 10 years.

HEPATITIS B VIRUS (HBV)

The discovery of the Australia antigen initiated a systematic research that eventually revealed that this antigen, originally considered an isoantigen, represented the surface protein of the HBV produced in excess as compared to the complete virions.\textsuperscript{21,22}

HBV continues to be the single most important cause of viral hepatitis throughout the world and is an important cause of chronic liver disease and hepatocellular carcinoma. HBV is a member of the family Hepadnaviridae and is the only human derived virus in the genus Orthohepadna virus.
Infection due to HBV is mainly through the parenteral, vertical and sexual transmission. The incubation period averages 75 days. The infectivity titre of HBV in blood may be $10^8$ or more infectious doses per ml.

It is a small hepatotropic DNA virus consisting of 3 main antigens, namely -

i) Hepatitis B surface antigen or HBsAg which represents the outer coat of the 42 nm virion also called the Dane particle. HBsAg is seen in tubular forms 20 nm in diameter and 100 nm long or in spheres.

ii) Hepatitis B core antigen or HBCAg: Treatment of the virus particle with the detergents leads to the release of the core particle which is known as HBcAg. While HBsAg circulates freely, HBcAg is not detectable in the serum.

iii) Hepatitis B envelope antigen or HBeAg. The detection of this antigen is important, as it reflects the replication of HBV, and in turn, a highly infectious state.

Genome of HBV

Inside the core of the virion is present the viral genome consisting of HBV-DNA and DNA-polymerase. HBV-DNA is partially double stranded and circular. It is approximately 3.2 Kb in length. The advent of DNA recombinant technology has allowed cloning and sequencing of the double stranded DNA genome of the HBV. Four open reading frames (ORFs) coding for putative
proteins have been identified. The ORF-1 consists of three regions, two for Pre-S and one for S gene which encodes for the surface antigen. The ORF-2 which has gene C codes for viral core polypeptide. Gene P which encodes for the putative DNA polymerase overlaps the S gene and is present in the ORF-3. The fourth ORF, namely the ORF-4 has the Gene X which is involved in transcription of HBV leading to the development of hepatocellular carcinoma.²⁶

Pattern of host response

HBV infection can cause a broad spectrum of disease ranging from asymptomatic infection to fulminant hepatitis. Those individuals with persistent infection can remain asymptomatic or progress to chronic liver disease and primary hepatocellular carcinoma.⁸

Methods of detection

The methods of detection include Serological Techniques and Molecular methods.

i) Serological Techniques

Corresponding to each antigen, there is antibody response namely, Anti-HBs, Anti-HBe and Anti-HBc. The 2 antigens, HBeAg and HBeAg together with the 3 antibodies can be detected in the serum by serological techniques. ELISA is extensively used owing to its higher sensitivity and specificity compared to the 1st and 2nd generation techniques.⁸
First generation - Agar Gel Diffusion
Second Generation - Counter Immuno electrophoresis
- Complement fixation test
Third Generation - Reverse passive Haemagglutination
- Enzyme linked Immunosorbent assay
- Radioimmuno assay

ii) Molecular Methods

These are useful for the detection of HBV-DNA and DNA-polymerase.

Molecular hybridisation has become one of the principal diagnostic techniques for the detection of viral infection. Transfer of total nucleic acids can be carried out by using a dot or slot apparatus. These are referred to as dot or slot blot hybridization assays, respectively.

Alternatively, this can be done according to molecular size by electrophoresis in an agarose gel before transfer onto a membrane. The assay is referred to as Southern blot if the nucleic acid is DNA.

One of the major advantages of this technique is the ability to measure the amount of viral genome present in the sample. The quantity of virus is a prognostic indicator of response to interferon therapy, the treatment currently recommended for chronic hepatitis.
Polymerase chain reaction is a relatively new and extremely sensitive technique and is $10^4$ times more sensitive than dot blot hybridization assays for the detection of HBV-DNA.\textsuperscript{27-29}

**Rational approach for the diagnosis of HBV infection in clinical practice**

| Acute Hepatitis B | - | HBsAg +, Anti-HBc IgM + |
| Fulminant hepatitis | - | HBsAg +/-, Anti-HBc IgM+, HBsAg containing circulating immune complexes+ |
| Chronic hepatitis B | - | HBsAg +/-, Anti-HBc IgM +/-, HBeAg +/- and Anti-HBe -/+ |
| Before antiviral therapy | - | HBV-DNA Quantification |
| Before Blood Donation | - | HBsAg |
| - | Role of IgM Anti-HBc, HBV-DNA? |
| Pre-vaccination screening | - | HBsAg |
| Post-vaccination screening | - | Anti-HBs titre should be checked $\geq 10\text{mIU/ml}$ protective titre |

There has been much interest recently in genetic variants of HBV and their impact on clinical disease. Variants of HBV with specific mutations within the precore and / or core region have been identified, principally in the Mediterranean region and Asia.\textsuperscript{30} These 'pre-c/c' mutants are thought to be associated in some cases with fulminant acute hepatitis and more severe chronic hepatitis.\textsuperscript{31,32}
A second type of HBV mutant virus containing one or more mutations in the S-gene encoding the envelope protein has also been reported. These ‘S-gene’ mutant viruses have been thought to represent neutralization escape mutants of HBV, since they have been recovered from individuals actively or passively immunized against the virus.

Prevention of Hepatitis B Virus infection

Vaccines

Because of the sharing of antigenic determinants between HBV and the 22nm-surface particles (HBsAg), plasma from asymptomatic carriers can be used as a source of immunogen

The virtual absence of in vitro culture systems for HBV stimulated considerable effort to develop safe and effective hepatitis B vaccines. In the 1970’s Krugman and colleagues demonstrated that serum containing HBsAg was inactivated after boiling and that this preparation was immunogenic and induced protective antibodies [Plasma derived vaccine].

These so-called ‘first-generation’ products have been largely replaced, however by HBsAg particles expressed in yeast, representing the first human vaccine product that has become available as a result of gene cloning technology. This is commercialised first by Smithkline Biologicals by name ‘Engerix-B’.
A study was conducted to evaluate the safety and efficacy of plasma derived (Heptavax-B) and genetic recombinant (Engerix-B) hepatitis-B vaccine. The observations revealed that both the vaccines are safe and immunogenic and these vaccines did not give rise to any recognizable post-vaccinial complications.

Prophylaxis

Pre-exposure prophylaxis

Hepatitis B vaccine is given intramuscularly (deltoid, not gluteal) at 0,1,6 month schedule. However for neonates, the vaccine is given in the lateral aspect of thigh to avoid chance injury to circumflex nerve. Studies have shown that a 20 µg dose is the most appropriate for adults and 10 µg for children and neonates.

Post-exposure prophylaxis

Hepatitis B immunoglobulin (HBIG) single intramuscular dose of 0.06 ml/kg is administered as soon as possible after exposure, followed by a complete course of Hepatitis B vaccine to begin within the first week.

For sexual contacts, HBIG 0.06 ml should be given within 14 days of exposure, followed by a full course of vaccine.
DELTA HEPATITIS

Hepatitis delta virus was discovered by Rizzetto et al., italy in 1977 and was initially described as a new antigen detectable in patients with chronic HBV liver disease. It is not able to replicate on its own, but is capable of infection when activated by the presence of hepatitis B Virus. HBV infection permits HDV infection to occur in two circumstances as HDV-HBV coinfection on an HBV-susceptible person and as HDV superinfection on an HBV carrier. The nature and consequences of these infections differ, primarily in that HDV superinfection of an HBV carrier usually leads to establishment of chronic HDV infection and chronic hepatitis. With co-infection, the acute delta hepatitis is usually self-limited, as the delta cannot outlive the transient HBs antigenaemia. About a third of fulminant hepatitis B is related to coincidental delta infection.

The HDV is a 35-38 nm enveloped particle that contains a small circular single-stranded RNA of about 500,000 daltons (1.7 kilobases), a unique internal protein (the delta antigen) and an outer coat of the hepatitis B surface antigen.

Serological tests to detect antibodies to the delta antigen provide the most useful diagnostic and epidemiologic tools. Both IgG and IgM antibodies develop in the course of natural HDV infection. Serum and liver HDV RNA are found in IgM anti-delta positive patients with acute and chronic hepatitis.
Non-A non-B hepatitis

Despite adoption of sensitive assays for screening of donor blood for HBsAg, anti-HBc and abnormal ALT levels, 7-10% of transfused patients (3-6 cases per 1000 units transfused) developed Non-A, Non-B Hepatitis (NANBH). NANBH was first described after blood transfusion.46 NANBH was felt to be responsible for about 90% of the cases of post-transfusion hepatitis in the United States. Although, most cases of post-transfusion acute NANBH are clinically silent, serious chronic liver disease (Chronic active hepatitis or cirrhosis) can occur in as many as 60% of cases.47-49

In contrast to this, chronic liver disease (CLD) was not observed after acute non-parenterally transmitted NANBH.50 The studies revealed the existence of at least two NANB viral agents, one of which is commonly associated with post-transfusion hepatitis associated with the development of CLD and the other whose main mode of transmission is enteric and which does not lead to chronic sequelae.

Enterically-Transmitted Non-A, Non-B Hepatitis

Non-A, Non-B Hepatitis

Parenterally-Transmitted Non-A, Non-B Hepatitis
Table 2.1

Historic perspectives in NANBH research since recognition of its existence

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Hepatitis C Virus

A third major category has always been suspected, but in the absence of a diagnostic test had been designated NANB viral hepatitis. This third type has now been identified and called Hepatitis C Virus.

Analysis of the HCV genome indicates that this virus is a new member of the third genus of the flaviviridae family. Viruses in this family are enveloped virion particles with positive stranded RNA as a genome. Flavivirus replication mainly occurs in the cytoplasm.

The incubation period of hepatitis C virus averages 50 days. It is commonly transmitted via blood and blood products. Its transmission by other routes, such as sexual, perinatal have been proposed but remain controversial and probably of minor importance.

The infectivity titre of HCV in the blood may be as high as $10^6$ infectious doses per ml but is usually much lower especially in cases of chronic infection, apparently because of complexing of virus to antibody.

Acute hepatitis C is generally a mild disease with a mortality rate of < 1%. However, > 50% of acute cases progress to chronicity, and some of these will eventually lead to cirrhosis or hepatocellular carcinoma or both.

In 1989, the First generation of anti-HCV immunoassays were introduced to detect viral infection. These assays detected antibodies that
reacted against a recombinant protein (C-100) derived from a non-structural region (NS 4) of the viral genome. During this period, more than 20% of NANBH were still unrecognised. The Second Generation immunoassays which include ELISA 2 and Recombinant immunoblot assay (RIBA 4), incorporated 2 additional recombinant proteins, one derived from the viral structural gene encoding the core antigen and the other derived from the non-structural region NS3 (also designated as C-33). The core and NS3 gene products have proven to be early seroconversion makers and could be readily identified in almost 95% of chronic NANBH.74

Antibody was detectable at approximately 22 weeks after transfusion of blood products (source of infection). However, the statistics have improved with the second-generation immunoassay and antibodies are detected 30-90 days earlier than with testing by first-generation assays.

Polymerase chain reaction detects HCV-RNA early in the course of HCV infection and sera that were seronegative for HCV antibodies have been shown to be positive for HCV genomic RNA by a highly sensitive RT-PCR assay.75-77

Atleast six major genotypes, which can be subdivided into over a dozen minor genotypes, have been identified and more are sure to be found. It is unclear how many serotypes of HCV exist, but failure of infection with one strain to protect against other strains or even the same strain make it likely that there are multiple serotypes of this virus.78-81
Attempts to isolate and propagate HCV in cell culture have met with limited success. The most successful results have been obtained with primary primate hepatocytes and various lymphoid cell lines. Whether or not these in vitro systems prove useful for replication of HCV, their greatest value may be in detecting and characterising neutralising antibody to the virus.

Despite these problems, research on HCV has yielded positive results. One of these is the control of transfusion-associated hepatitis C. The new and constantly improving screening tests for HCV infection, in conjunction with the already widely used screening tests for HBV and HIV infection, have almost eradicated transfusion-associated hepatitis due to these viruses in developed countries.16

ENTERICALLY TRANSMITTED NON-A NON-B HEPATITIS

An agent for enterically transmitted Non-A, Non-B Hepatitis (ET-NANBH) was not recognized as a unique human disease until 1980, when serologic tests for the diagnosis of hepatitis A and hepatitis B were applied to stored clinical samples collected during water-borne epidemics of viral hepatitis in India. Among these was the massive epidemic of hepatitis that occurred in Delhi, India in 1955-56 following contamination of a major water treatment plant with raw sewage.82 The epidemic had been cited previously as a classical example of water-borne hepatitis A, but the subsequent discovery that hepatitis A was highly endemic in Indian population, with HAV infecting almost 100% of the population by the age of 5 - 10 years made it difficult to
accept that the Delhi epidemic and other water-borne epidemics that occurred principally in young adults were caused by HAV.

Indeed, virtually 100% of stored serum samples from such epidemics were found to contain anti-HAV IgG but not anti-HAV IgM - strong evidence for past HAV infection with resultant immunity and therefore evidence for the existence of a previously unrecognised hepatitis agent as the cause of epidemics.

Subsequently in 1983, the finding of 27-34 nm virus-like particles (VLPs) in stool specimens of acutely infected cases of non-A, non-B hepatitis were reported.61

However, definitive studies linking these VLPs to ET-NANBH have been prevented by the absence of a suitable primate model and by the lack of reagent including partially purified virus and disease-specific antibodies.

HEPATITIS E VIRUS

Hepatitis E virus (HEV) is the name proposed for the virus or group of serologically related viruses that cause enterically transmitted non-A, non-B hepatitis in an epidemic as well as in a sporadic setting.6 This epidemic form of non-A, non-B hepatitis appears to pose major public health problems in most parts of the world particularly in developing countries and especially in India.
Morphology

Size: The virus ranges in diameter from 27-34 nm with a mean of 32-34.

Structure: The virus is spherical and unenveloped with spikes and indentations visible on the surface of the particle.

Biophysical properties: Rate-zonal banding of HEV in linear, preformed sucrose gradients was found to yield partially purified virus (32-34 nm particles) suitable for further studies. The computed sedimentation coefficient for HEV was approximately 183 S.61,63,83

Resistance to environmental factors

It has been repeatedly noted that unlike HAV, HEV is extremely labile under laboratory conditions and often cannot be recovered from the test solutions after routine procedures such as freeze-thawing, treatment with lipid solvents and pelleting.

Unfortunately, the physical or chemical nature of factors affecting HEV integrity in these conditions remains obscure. This property of the virus obviously conflicts with the main epidemiological feature of HEV infection, i.e. faecal-oral mode of spread; transmission through contaminated water or food depending upon an infectious agent which is relatively stable in the environment.84
It is not known how to kill or attenuate the virus, how to treat water to reduce or eliminate the risk of infection or whether human immune serum globulin can be used for prophylaxis of infection.

Classification of hepatitis E virus on the basis of phylogenetic analysis

HEV is the causative agent of the ET-NANBH an epidemiologically important disease. HEV has naked, icosohedral virus particles resembling virions of calicivirus. Moreover, the gene organisation of the 7.5 kilobase, single-stranded RNA genome of HEV also resembles that of calicivirus, with the capsid protein gene at the 3' -end and the gene encoding the non-structural polyprotein at the 5' end. On the basis of these similarities, HEV is currently included in the family caliciviridae.

However, the sequence comparisons of the non-structural proteins show that HEV is related not to caliciviruses but to rubella virus (Rub V) and the beet necrotic yellow vein virus (BNYVV) which belong to the alpha-like supergroup of positive-strand RNA viruses. HEV, Rub-V, BNYVV and alphaviruses share an array of conserved domains which includes RNA-dependant RNA polymerase, RNA helicase, methyltransferase and papain-like cysteine protease. An additional domain with unknown function is conserved only in HEV, Rub V and BNYVV. Phylogenetic analysis of the RNA-dependent RNA polymerase and the RNA helicase sequences show that HEV, Rub V and BNYVV form a compact group within the alpha-like supergroup. These findings constitute a basis for re-classification of HEV. HEV may be considered
the prototype of a new virus family, perhaps within a larger taxon that would bring together HEV, Rub V and BNYVV.²⁶

**Analysis of the HEV genome**

Molecular cloning of HEV revealed it to be a single-stranded, positive-sense RNA virus of about approximately 7.5kb in length with 3 forward open reading frames. The virus has an apparent 5'-non-structural-3'-structural genomic organization. Its expression strategy involves the utilization of portions of all 3 forward reading frames, with some of the 3' end viral gene products presumably transcribed within 2 subgenomic RNA transcripts.

The open reading frame (ORF) 1 which consists of 1693 codons and is the largest of the three ORFs, encodes for the non-structural proteins with 7 domains namely the methyl transferase, domain "Y", X domain, cysteine protease, helicase, a polyproline region that may act as a flexible hinge and RNA - dependent RNA polymerase. The presence of methyl transferase suggests that the HEV genome should be capped. These 7 domains encompass most of the HEV non-structural polyprotein and with subsequent proteolytic processing, may be contained in four or five distinct proteins.

The HEV ORF 2 which consists of 660 codons appears to encode the structural protein (s) of the virus. The amino terminus of ORF 2 contains a signal peptide. It is not known if ORF 2 encodes multiple proteins or a single protein as seen in Norwalk and feline calici virus. Bile was used for the
construction of recombinant complimentary DNA (cDNA) libraries. Immunoscreening of lamda gt 10 and gt 11 libraries with sera from well-documented HEV outbreaks and infected cynomolgus macaques led to the isolation of 2 different cDNA clones. One of these immunodominant clones came from the extreme 3' end of ORF 2. All these data indicate that ORF 2 possibly encodes the structural protein(s) of HEV.

The second cDNA clone isolated by immunoscreening came from a small third ORF that overlapped ORF 1 and ORF 2. This ORF contained 369 nucleotides and therefore had the capacity to encode a protein of at least 123 amino acids.64

Clinical course of infection

The liver appears to be the only target organ for infection and extrahepatic manifestations of infection have been rare. The incubation period, as determined from epidemic situations, ranges from 15 - 40 days, with the average being longer than that observed with hepatitis A. The preicteric phase lasts for 1 - 10 days (average 3 - 4) and gastrointestinal symptoms such as epigastric pain, nausea and vomiting are frequently reported. The icteric phase begins abruptly with the appearance of jaundice, dark urine and clay-coloured stools. In uncomplicated cases this last 12 - 15 days and complete recovery usually takes place within one month.
Clinical studies have shown that elevations of serum alanine aminotransferase (ALT) levels occur as a single peak preceding or coinciding with the onset of jaundice,\textsuperscript{61,87-89} which is similar to most other forms of viral hepatitis.

Experimental HEV infection in non-human primates has consistently produced a biphasic pattern of ALT elevation, with a minor peak observed 6 - 10 days after inoculation and the major peak at the time of histopathologic liver injury.\textsuperscript{90-93} The observation of the first ALT peak in patients would be rare, since clinical chemistries are rarely obtained during the preclinical stage of infection. Resolution usually occurs within weeks and there is no evidence of chronicity. Pregnant women are at higher risk for developing fulminant disease and third trimester fatality rates up to 32\% have been reported.\textsuperscript{6,94-100}

The high mortality from fulminant hepatitis in pregnant women is particularly striking and high incidence of disseminated intravascular coagulation associated with the disease in pregnant women has been noted. Schwartzman's phenomenon has been proposed as a unifying theory for the pathogenesis of fulminant hepatitis E in pregnant women. The hypothesis is as follows: damage by the virus to the sinusoidal cells, particularly the kupffer cells, diminishes their ability to protect the hepatocytes against endotoxins originating from gram negative bacteria in the intestinal tract. Direct injury of hepatocytes by endotoxin and secondary injury, mediated through release of eicosanoids, leads to prostaglandin-mediated chemotactic attraction of
neutrophils and leukotriene-mediated oedema and cholestasis. The enhanced sensitivity of pregnant women to such an endotoxin mediated effect is well recognised and may explain the striking mortality of Hepatitis E in pregnancy. The validity of this theory is yet to be tested by careful clinical evaluation of affected pregnant women in epidemics of the disease.101,102

Etiopathogenesis

The pathogenesis of HEV infection has been learned mostly in experimentally infected non-human primates. Much information is not available about the pathogenesis in humans.

The major mode of transmission is through the enteric route and the incubation period of the virus averages 40 days.

Presumably HEV enters the blood from the enteric tract, replicates in the liver, is released from hepatocytes into the bile and is subsequently excreted in the faeces; viraemia has only been reported recently.103 However, there is no evidence for a long-term carrier state.

Analysis of serum specimens collected from HEV patients during the incubation period, acute phase and convalescence have shown the order of appearance of the various diagnostic molecules. The usual sequential order is HEV-RNA in stool, HEV-RNA in serum, anti-HEV IgA/IgM and anti-HEV IgG.
A study was conducted to determine the pattern of ALT activity, viraemia, faecal shedding and antibody response in patients with hepatitis E. The data were analysed with respect to the time after onset of illness:

Mean ALT activity was highest in sera collected 0 - 3 days after onset and declined thereafter, suggesting that hepatocyte injury is greater at onset of illness than afterwards.

The most remarkable result of the study was that viremia was detected in more patients than was faecal shedding or an antibody response. HEV-RNA was detected in 71% of sera collected in the first 3 days of illness. However, it increased with time reaching a maximum of 91% at 8 - 11 days suggesting that viral replication peaks several days after the onset of illness.

The finding that the proportion of RNA positive sera increased from days 0 - 3 to days 8 - 11 in the presence of decreased ALT activity suggests an uncoupling of viral replication and liver injury. The detection of prolonged viraemia upto 5 weeks (in some patients) in the presence of antibodies to structural protein epitopes raises the possibility that acute-phase immunoglobulin may lack neutralizing activity, although this has not been demonstrated. In any event, immune complexes may circulate in hepatitis E patients. The possible role of immune complexes in the pathogenesis of hepatitis E should be examined.
Replication of HEV

It has been shown earlier that HEV can be passaged serially in animal models and HEV antigen could be demonstrated in the hepatocytes of experimentally infected cynomolgus macaques. This provides indirect evidence of viral replication in the livers of experimentally infected animals. But, there are some viruses that replicate at one site and cause disease at another site in the host. Therefore, it was important to document directly replication of HEV in the primary organ that it infects.

HEV was postulated to replicate through a negative-stranded RNA intermediate based on the presence of a genomic positive-stranded RNA and a putative RNA polymerase domain characteristic of positive-sense RNA viruses. The postulate was confirmed by the detection of the negative strand of HEV-RNA in the livers of experimentally infected rhesus monkeys.

The ability to detect HEV negative-stranded replicative intermediate will provide a useful assay to monitor the growth of HEV during the in vitro cultivation of the agent.

Hepatic pathology

Specimens of liver tissue have not been available from more recent hepatitis E outbreaks. The most extensive histopathological studies were carried out in liver specimens from 78 patients during the outbreak in Delhi.
in 1955-56,\textsuperscript{119} from 31 patients from the Kashmir epidemic in 1978-79,\textsuperscript{50} from 128 patients with endemically occurring hepatitis E in Ghana, West Africa in 1962-63\textsuperscript{110} and from a pregnant women with fulminant hepatitis in Kathmandu, 1990.\textsuperscript{111}

Morphologic features of hepatitis E include those of cholestatic and 'standard' (classic) types of acute viral hepatitis.

**Light microscopy**

The histological picture in AVH-E are described as ballooning of hepatocytes and focal hepatocyte necrosis; pseudoglandular alteration of hepatocytes; cytoplasmic and canalicular cholestasis; acidophilic degeneration and acidophil bodies. Inflammatory infiltration showed mononuclear macrophages, lymphocytes and Kupffer cell proliferation.\textsuperscript{111,112}

In the fulminant case of hepatitis E, liver tissue showed necrosis of parenchyma with collapse of liver lobules and condensation of connective tissue. Remaining hepatocytes were swollen and had a foamy appearance. They were scattered as single cells and in groups or were arrayed in a gland-like fashion around distended bile canaliculi filled with bile plugs. There was extensive proliferation of small bile ductules. Branches of the portal vein and the central veins showed signs of phlebitis with edema and inflammatory infiltrate. Lobules contained predominantly macrophages and
polymorphonuclear leucocytes. The kupffer cells were prominent and contained PAS-positive, diastase-resistant cytoplasmic granules indicative of lipofuscin.\textsuperscript{94}

**Electron microscopy**

Extensive proliferation of small bile ductules and biliary epithelia that contain bundles of filaments are seen. Fatty metamorphosis is seen, as are bile accumulations and an increased number of phagolysosomes. Hepatocytes exhibit hydropic degeneration and severe damage of all cellular organelles. In one patient, the perinuclear membrane of cells contained numerous vesicular structures with round and irregular shapes, measuring about 45-55 nm in diameter, which were electron lucent. Similar structures, measuring about 40 nm, were also seen in the cisternae of rough endoplasmic reticulum connected with the perinuclear membrane.\textsuperscript{94,112}

**Experimental transmission of infection**

On two occasions, transmission of infection to human volunteers has been reported with inocula (filtrates of faeces from patients with the disease) from different outbreaks.\textsuperscript{61,66}

Faecal specimens obtained from these patients during the acute phase of their illness and single specimens or pools of specimens obtained during outbreaks in several countries have been inoculated into a wide range of animals which include monkeys, chimpanzees, pigs and laboratory mice.
A variety of factors have been used to determine whether the animal has been infected and no agreed criteria exist. Because most "infections" in non-human primates have been diagnosed on the basis of mild, transient elevations in serum transaminase or dehydrogenase levels, it is essential that animals used in these studies be free from other infectious agents (especially parasites) and observed for a prolonged period before inoculation.

In animals inoculated with a single specimen, several or all of the following criteria should be met before accepting that transmission has occurred:

1) Serum enzyme levels should increase to at least twice the mean of preinoculation values on at least two consecutive occasions.

2) Increases in liver enzymes should be accompanied by evidence from biopsy, of hepatitis or some characteristic morphological or ultrastructural change.

3) Specific antigens or viral particles should be detected in the animal's liver and/or faeces at appropriate stages of the illness.

4) Examination of preinoculation and convalescent phase sera should demonstrate a seroconversion of antibodies to this antigen or virus particle.
5) It should be possible to transmit these effects to additional animals by inoculating them with an extract of faeces or liver collected during the acute phase of the illness.\textsuperscript{113}

Studies in primates

Earlier studies used cynomolgus macaques which were intravenously inoculated with a 10\% w/v suspension of a human volunteer stool that was positive by IEM for 27-30 nm diameter VLPs. These animals developed elevations in ALT activity between 24 and 36 days after inoculation, excreted 27-30 nm VLPs in their pre-acute phase stools, and seroconverted to morphologically similar VLPs in the inoculum.

Later, in 1986, successful transmission of ET-NANBH was reported in African green monkeys, as well as in cynomolgus macaques using ET-NANBH case stool specimens positive by IEM for 27-30 nm VLPs.\textsuperscript{114} Although the agent for ET-NANBH was serially passaged in the latter animals, a decreased efficiency of transmission was observed during each successful passage. After passage the disease has become slightly milder, and the incubation period has been reduced from six to three weeks.\textsuperscript{113}

An important breakthrough was the recognition that gall bladder bile, collected at necropsy from a cyno-infected with a Burma derived, third-passaged, ET-NANBH inoculation, contained VLPs by IEM.\textsuperscript{86} It was previously empirically determined that differential hybridization (plus-minus screening)
had a detection sensitivity of about 1 : 1000 (target to background ratio\textsuperscript{115} and it was postulated and later proved\textsuperscript{86} that the lower nucleic acid sequence complexity present in bile, when compared with other potential cloning sources such as faeces, serum or liver might have the required sensitivity to detect a unique class of viral origin. However, the problem of low titer of HEV in clinical samples was overcome by the technique known as Sequence-Independent, Single-Primer Amplification (SISPA)\textsuperscript{116} The detection of negative-strand of HEV replicative intermediate in the livers of experimentally infected rhesus monkey validates the use of this animal model to study the pathogenesis of HEV\textsuperscript{108}

Studies in chimpanzees

Inoculation of chimpanzees with ET-NANBH case stools obtained from outbreaks in geographic areas other than Mexico, or containing either fewer numbers of 32-34 nm VLPs (or smaller VLPs) demonstrated less severe disease or no disease at all. The stools used in these studies were derived from cases of ET-NANBH occurring in India, USSR and Burma.\textsuperscript{117} These studies also reveal the possibility of parenteral transmission of HEV, though it may play a minor role due to the transient presence of HEV-RNA in the blood.

Studies in domestic swine

Successful transmission of HEV infection to domestic pigs (Sus scrofa domestica) have been reported.\textsuperscript{118} Intravenous inoculation of 10\% stool extract
resulted in biphasic elevation of transaminase at 7-11 days and 16-23 days postinoculation in two of the four experimental animals. The other two showed a monophasic pattern and all the animals had shed HEV particles in stool from 6 - 20 days post-inoculation as detected by IEM.

A noteworthy feature is that these animals developed jaundice with characteristic icteric skin, particularly in the axillary fossae as well as yellow-coloured sclera observed distinctly during weeks 3 and 4 after inoculation, which was not demonstrated previously in non-human primates inoculated with any human hepatitis virus.

Detection of natural infection in swines (Sus scrofa Yorkshire, Sus scrofa landrace, Sus scrofa vitalis) as evidenced by HEV-RNA in blood, stool and detection of IgM and IgG in the sera indicates that HEV is a zoonotic virus.\textsuperscript{119}

Studies in laboratory rats

Recently, experimental infection of the laboratory rats (Wistar rats) with HEV, confirmed that HEV can replicate in them. The route of inoculation was intravenous. HEV antigen was detected in liver, peripheral blood mononuclear cells, spleen, mesenteric lymph nodes and small intestine. Histopathology attributable to the inoculum were seen in liver, spleen and lymph nodes.\textsuperscript{120} The study suggests new tissue sites for HEV replication.
Cultivation of HEV

The development of diagnostic tests for the detection of HEV infection was hampered for a long time, owing to a number of reasons, the most important being the non-availability of large amounts of viruses.

This was due to two reasons:

a) Detection of virus in the faeces after the onset of clinical symptoms is only 30%.

b) Numerous attempts to grow this virus in cultivated cells using conventional cell culture inoculation techniques have proved unsuccessful. However, HEV was detected in serial passages of fresh foetal rhesus monkey kidney (FRh K-4) cells after they had been co-cultivated with primary kidney cells derived from cynomolgus monkeys experimentally infected with this virus.\(^{121}\)

However, the 87A strain isolated in 2BS cells from the faeces of a patient with hepatitis E, was propagated in A549 cells and the marked cytopathic effect appeared in the infected monolayer cells. The size of this virus was about 30 nm in diameter. Furthermore, HEV-RNA from the supernatants of different passages was detected by PCR amplification using ET 1.1 HEV primers.\(^{122}\)
Recently, a tissue culture system using a serum-free medium formulation has been developed to propagate the virus in vitro. Hepatocytes were isolated from livers of cynomolgus macaques experimentally infected with a HEV (Burma strain) inoculum and maintained in long-term cultures using a highly strand specific RT-PCR assay. Both the positive-sense and the negative replicative strands of HEV-RNA were detected in these hepatocytes throughout the course of the experiments. Positive-strand genomic RNA was also detected in the culture medium, suggesting the production and secretion of HEV virus particles. The virus particles were successfully concentrated 200-fold from the medium using ultrafiltration and they could be observed by IEM using anti-HEV positive immune serum. These results demonstrate the capacity of this hepatocyte culture system to replicate HEV in vitro, thus providing an experimental means to study the replicative process of the virus.\textsuperscript{123}

Identification of HEV infection

Cloning of HEV, sequencing of the viral genome and expression of recombinant HEV proteins facilitated significant progress in the development of methods for identification of HEV infection in patients and experimental animals. Diagnosis of HEV infection was based on -

a) Tests that detect the Hepatitis E Virus and
b) Tests for detection of antibody to HEV (anti-HEV) in serum.
Tests that detect the Hepatitis E virus

Electron Microscopy studies

HEV particles measuring 27-34 nm in diameter regularly recovered from stools of infected animals before or at the beginning of aminotransferase level elevation, were identified by Transmission electron microscopy.\(^{61}\)

VLPs similar to those detected in the Soviet Union were subsequently detected by other laboratories and serologic cross-reactivity was demonstrated between VLPs from geographically different epidemics of ET-NANBH.\(^{6,63,90,117,124,125}\)

The range of size of HEV particles identified in stool preparations by various laboratories was not uniform because the indefinite outline of viral particles coated by antibody made precise measurement difficult. 27-30 nm VLPs were first recovered from the stools of patients in Tashkent, Uzbekistan from a human volunteer who contracted hepatitis E when infected with a stool extract from these patients and from stools of experimentally infected cynomolgus macaques.\(^{61}\)

Further experimental studies revealed that VLPs derived from stools and bile of infected animals varied in size from 27 to 32 or 34 and rarely even 38 nm (mean diameter - 32.3 nm) The particles were spherical and non-enveloped with indentations and spikes on their surfaces similar in appearance to those of caliciviruses. The particles were identified on IEM when they were
coated and aggregated by acute-phase sera or serum pools from hepatitis E outbreaks in various geographic locations.\textsuperscript{63} IEM was the reliable method and highly specific tool for the detection, identification and characterisation of agents not cultivable in an invitro system.

The variabilities in the size may also be related to proteolytic digestion of HEV in its passage through the gut, to its sensitivity to freeze-thawing or to storage of stool preparations. The possibility of proteolytic degradation of the virus is consistent with the unexpectedly low numbers of HEV particles found in acute-phase stools from patients with hepatitis E. The frequency of finding VLPs by IEM with the use of relatively high titre antiserum is not more than 10\%. Most positive results would be from cases within first five days of onset of symptoms.\textsuperscript{117}

Detection of hepatocellular HEV Ag by immunofluorescence

HEV Ag has been identified in the cytoplasm of hepatocytes of experimentally infected macaques, monkeys and chimpanzees with an immunofluorescent probe prepared from convalescent-phase serum aggregating 27-34 nm HEV particles.\textsuperscript{107} The specificity of the HEV Ag immunofluorescence was determined in a series of absorption and blocking experiments. Reactivity of the fluorescein-labelled anti-HEV Ag with HEV Ag in hepatocytes was blocked by acute and convalescent phase serum samples, but not by serum samples obtained before infection.
HEV Ag was located randomly throughout the cytoplasm of hepatocytes and the number of fluorescent granules of antigen varied (sometimes only a few fluorescent granules were visible through a cell). Most granules were uniform in size; slightly larger granules were rarely observed. Hepatocytes containing HEV antigen were frequently found in groups forming indistinctly outlined foci of positive cells located in central, midzonal or periportal parts of liver lobules. In highly positive specimens, 70-90% of hepatocytes were positive for HEV Ag.111

Correlations of the presence of HEV Ag in hepatocytes with the presence of HEV genomic sequences in stool and serum, with histopathological changes in the liver and with humoral and cellular antiviral immune responses during the incubation and replicative phases of HEV infection will contribute to studies of the natural history of hepatitis E.

Reverse-transcriptase polymerase chain reaction

The development of PCR by Kary Mullis while at the Cetus Corporation has made enormous progress in HEV because of the very low levels of the circulating virus which cannot be detected by other detection systems. HEV-RNA sequences were identified in stool and serum samples from patients and experimentally infected primates. Amplified HEV sequences were those located in a putative polymerase region of ORF 1 or at the 3' end of ORF2.111,119,126-129
b) Tests for detection of antibody to HEV

This can be grouped under 3 heads depending on the use of -

1) Native HEV antigens
2) Recombinant HEV antigens and
3) Synthetic peptides.

Tests that use native HEV antigens

Identification of anti-HEV against native HEV antigens has been carried out with IEM and a fluorescent antibody-blocking assay using preparations of HEV particles or sections from infected livers containing HEV Ag respectively.

IEM showed that geographically distinct viral isolates reacted with sera from various hepatitis E outbreaks and that animals infected with one isolate readily acquire antibody reactive with particles of another geographically distinct isolate.

IEM while very specific has a number of disadvantages as a diagnostic tool. It requires highly trained observer, a great deal of time and a large amount of antigen and antibody.

A fluorescent antibody competition assay for the identification and titration of antibodies against native HEV Ag was carried out on liver sections
containing noticeable amounts of HEV Ag in hepatocytes obtained from
cynomolgus macaques experimentally infected with HEV.\(^{130}\)

The anti-HEV immunological relationship between sera obtained from
geographically isolated outbreaks of hepatitis E was confirmed by the
seroconversion of primates infected with HEV isolates from Burma, Pakistan
or Mexico. Anti-HEV was found on antibody-blocking assay in macaques in
each of five passages of hepatitis E in tamarins and in two passages of HEV
infection in chimpanzees.\(^{111}\)

**Tests that use recombinant HEV proteins Western blotting**

Recombinant HEV proteins derived from Burmese or Mexican isolates
were used in anti-HEV immunoassays such as immunoblotting. The western
blot anti-HEV was also used for studies of outbreak and sporadic cases of
hepatitis E in the central Asia, Republics of Tadzikistan, Turkmenistan,
Kirghizstan and Uzbekistan. Anti-HEV IgM was detected in 73% of patients
within 26 days of onset of jaundice. In the same study, acute sporadic cases of
hepatitis E (76%) were identified among a small number of cases from the
Central Asiatic republics where HEV infection is endemic. The Western blot
assay was also used for identification of hepatitis E among sporadic acute
hepatitis cases in children (2-14 year old) living in an urban region of
Sudan.\(^{131}\)
Enzyme-linked immunosorbent assay

Due to their ease of use and potential ability to distinguish acute HEV infections from convalescent, ELISAs are now being utilised to determine the incidence of viral hepatitis due to acute HEV and the seroprevalence of anti-HEV in various geographical areas.

The development and evolution of a prototype diagnostic assay for HEV infection which is described as highly sensitive and specific for both IgG and IgM anti-HEV is reported. The investigators reported that antibodies to SG3, the carboxyl half of ORF-2 of HEV, was the most frequently detected. Anti-HEV IgG was detected in 9 of 268 (3.4%) and 2 of 168 (1.2%) of randomly selected volunteer blood donors in California and Texas respectively. In countries where HEV is endemic, 7 - 17% of the population were reactive for anti-HEV IgG. In cases of acute HEV infection, IgM and IgG antibody responses were confirmed by the presence of HEV-RNA by PCR.

Later in 1992, an EIA for the detection of IgA, IgG and IgM specific anti-HEV was developed utilizing two HEV recombinant proteins derived from the Burma strain of HEV. These included protein 8-5 (full length ORF-3) and protein SC3 (the carboxyl half of ORF 2).
Tests that use synthetic peptides

ELISA

Assays employing synthetic peptides have also shown promise for detection of anti-HEV. These assays were developed utilizing the ORF2 and ORF3 region of the HEV genome.¹⁰³,¹³²,¹³³

EPIDEMIOLOGY

The epidemiology of infectious diseases is concerned with the circumstances under which both infections and disease occur in a population and the factors that influence their frequency, spread and distribution. This concept distinguishes between infection and disease because the factors that govern their occurrence may be different and moreover infection without disease is common with many viruses.

The AGENT, the ENVIRONMENT and the HOST RESPONSE TO INFECTION are the most important determinants for any infection.

The agent

The spread of any virus depends on

a) The Stability of the virus within the physical environment required for its transmission, including resistance to high or low temperatures
Under experimental conditions, HEV virus is extremely labile. It is sensitive to freeze-thawing, storage in liquid suspensions at temperatures below -70 degrees and +8 degrees and pelleting from solutions of sucrose or buffer.

The low secondary attack rate among exposed household member when compared to HAV indicates that the virus is not stable under natural conditions also.92

b) The amount of virus expelled into the proper vehicle of transmission

This is an important factor as HEV virus is an important cause of epidemics of AVH. Such epidemics are usually preceded by massive contamination of the water supplies in which conditions the quantum of infective dose is very high.

The largest of the recorded outbreaks of HEV were in Delhi (1955-56)82 and Kanpur (1991)134 and are due to gross faecal contamination of the drinking water supplies.

The environment

A conducive environment is essential for the spread of infection as it exerts its influences on the agent itself and on the nature of the host response to infection.
For viral diseases potentially transmitted by water, such as hepatitis E, a warm environment attended by poor sanitation and faecal contamination clearly enhances the degree of exposure and the efficiency of transmission.

The host

The host responses to viral infections vary along a biological gradient in terms of both the severity and the nature of the clinical syndrome produced.

The biological gradient

The host response to a virus may range from a completely inapparent infection without any clinical signs and symptoms to one of great clinical severity, even death.

The biological gradient of host response is often pictured as an iceberg in which clinically apparent illness - i.e. above the waterline - represents only a small proportion of the response pattern and the larger amount represents unrecognized and inapparent infections; a similar analogy may exist at the cellular level.
Some of the factors that influence the clinical host response

1. Dosage, virulence and portal of entry of the agent

The incubation period of the virus which averages 4 weeks has been found to be low during epidemics, probably because the quantum of infective dose is higher in such conditions, as there will be massive contamination of water supplies.

Human volunteer studies indicate that the transmission of HEV can occur primarily via the enteral route.61,66

2. Age at the time of infection and sex

HEV infection predominantly occurs in young adults preferably in the second and third decade of life. Recently, HEV has been proved to be the most common cause of acute sporadic hepatitis in paediatric populations in endemic regions.131

3. Nature and vigour of the immune response

The immune response is weak and transient. Anti-HEV IgM can be detected from 7 - 10 days after onset of symptoms to 3 - 6 months. Though the period of persistence of IgG antibodies is debatable, infection by HEV appears to induce no long term protective immunity.
Transmission studies in cynomolgus macaques reveal that in the absence of seroconversion, an additional bout(s) of hepatitis due to HEV can occur.91

4. Pre-existing level of immunity

It is not clear if the antibody response produced as a result of HEV infection imparts any protection against re-infection.135

This postulate was found to be true by the same workers by their animal transmission studies in Macaca mullata monkeys. Of the four monkeys used for the experiment, one animal was re-challenged with the original source material more than a year after the first inoculum when his serum enzymes and liver morphology had long returned to completely normal. The animal again showed marked enzyme elevation accompanied by re-appearance of the previous hepatocytic damage.

Pregnancy is an important factor that increases the mortality rate in viral hepatitis especially due to HBV and HEV and is associated with premature delivery and a high maternal and foetal mortality.95,96

5. Presence of receptor sites and cell-to-cell spread

Since extrahepatic manifestations of the virus are very low, the receptor should be present in the hepatocyte.
6. **Socio-economic status**

A high incidence of infection due to enterically-transmitted hepatitis viruses due to HAV and HEV are observed in lower socio-economic groups as the standards of hygiene are very low.

7. **Pre-existing conditions**

Epidemiologic data have suggested that hepatitis E can occur as a super-infection in chronic carriers of hepatitis B. In a study conducted in India, 32% of cases of presumed hepatitis E were associated with the HBsAg carrier state. However, there are differences in observations regarding the different clinical course of acute hepatitis E in HBsAg carriers from those without HBsAg.

It is usually recorded that morbidity from hepatitis in diabetics is 2 - 4 times higher than general population and that the course is more often protracted. Diabetes mellitus may be unmasked by viral hepatitis and both hypoglycemia as well as hyperglycemia can be a factor for coma in occasional instances.

Hypoglycemia is a terminal event and it is difficult to control in 10% of the patients in epidemics due to infective hepatitis.
8. **Personal habits**

Alcohol is hepatotoxic and can aggravate the course of viral hepatitis clinically, biochemically and histopathologically. Markers of past or current hepatitis B or C are commoner in patients with alcoholic liver disease than in the general population.

Undue physical exertion must be avoided in all types of AVH.

9. **Double infection**

Co-infection of HEV with other viruses, the commonest being its co-infection with HAV leads to a poorer prognosis and is important cause of acute liver failure in children.

**Global pattern**

The disease is endemic in a broad region encompassing much of S.E.Asia, Central Asia, Eastern, Northern and Western Africa and N.America. Recent surveys have shown that HEV is endemic in countries like Hong Kong, Egypt, Turkey, Taiwan, Netherlands, Italy and Spain. However, epidemics have not been reported in these countries.
Epidemic behaviour of HEV

The dramatic nature of the epidemics produced by this agent gave rise to its initial designation as epidemic non-A, non-B hepatitis. The first largest epidemic occurred in New Delhi, India in 1955-56 with an estimated 29,000 icteric cases occurring, following gross faecal contamination.

Nearly 40 epidemics of water-borne hepatitis due to ET-NANBH have occurred throughout India which were identified as ET-NANBH by exclusion of HAV and HBV. However, after the subsequent development of tests to detect specific markers for HEV infection, retrospective study on the stored samples have confirmed 18 epidemics to be due to HEV.

Reported epidemics are listed in Table-2.2 and depicted in Fig.2.1.
Table 2.2

Recorded outbreaks of infectious Hepatitis in India

<table>
<thead>
<tr>
<th>Place</th>
<th>Year</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Jammu and Kashmir</td>
<td>1912</td>
<td>Franklin, 1913\textsuperscript{140}</td>
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<tr>
<td>Agra</td>
<td>1949</td>
<td>Wani, 1953\textsuperscript{141}</td>
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<tr>
<td>Delhi</td>
<td>1955-56</td>
<td>Viswanathan, 1957\textsuperscript{82}</td>
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<td>Bombay</td>
<td>1959</td>
<td>Patel and Rao 1960\textsuperscript{142}</td>
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<td>Bihar</td>
<td>1959</td>
<td>Seal \textit{et al.}, 1960\textsuperscript{143}</td>
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<td>Guwahati</td>
<td>1960</td>
<td>Lyngdoh, 1969\textsuperscript{144}</td>
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<tr>
<td>Kharagpur (W.Bengal)</td>
<td>1960</td>
<td>Bhattacharji \textit{et al.}, 1963\textsuperscript{145}</td>
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<td>Aurangabad</td>
<td>1961</td>
<td>Dhamdhere, 1962\textsuperscript{146}</td>
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<td>1966</td>
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<td>Saxena, 1978\textsuperscript{149}</td>
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<td>Siliguri (Assam)</td>
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<td>1970</td>
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<td>Ahmedabad</td>
<td>1975-76</td>
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<td>Kashmir</td>
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<td>Mehta \textit{et al.}, 1981\textsuperscript{156}</td>
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<td>Arankalle et al., 1994&lt;sup&gt;67&lt;/sup&gt;</td>
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<td>1990</td>
<td>Charuprakash, 1991&lt;sup&gt;160&lt;/sup&gt;</td>
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<tr>
<td>Rainagar (Orissa)</td>
<td>1990</td>
<td>Bora et al., 1993&lt;sup&gt;161&lt;/sup&gt;</td>
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<td>1990</td>
<td>Arankalle et al., 1994&lt;sup&gt;67&lt;/sup&gt;</td>
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<tr>
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<td>1990</td>
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Fig. 2.1

RECORDED OUTBREAKS OF INFECTIOUS HEPATITIS IN INDIA

Scale 1:17,000,000 1cm = 170 kms

*Confirmed as due to HEV

Figures in parentheses indicate references
Outbreaks of HEV occur in a unimodal fashion with a highly compressed curve of incidence or as more prolonged epidemics with multiple peaks of incidence. In both instances, the source of the disease is usually contaminated water and epidemics are frequently observed after the rainy season. The secondary attack rate among exposed household members is low but significantly higher than the incidence of disease among non-exposed control households (7.7 Vs. 1.3 per 1000 population in Rangoon outbreak).\textsuperscript{111}

**Viral hepatitis in pregnancy**

For mammalian pregnancy to succeed, large physiological adjustments are required in the mother: these changes result from signals passing between the conceptus (especially the trophoblast) and the mother throughout pregnancy.

Every system in the body undergoes certain changes. Some of the changes in liver function related to normal pregnancy are:

<table>
<thead>
<tr>
<th>Test</th>
<th>Usual range during pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma volume</td>
<td>Increased greater than 50%</td>
</tr>
<tr>
<td>Blood volume</td>
<td>Increased to 40 - 50%</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>1.5 to 4 fold increase</td>
</tr>
<tr>
<td>Transaminases (ALT, AST)</td>
<td>Unchanged or slightly increased to upper limits of normal</td>
</tr>
</tbody>
</table>
Bilirubin - Unchanged or slightly increased
Prothrombin time - Unchanged
Factor 7-10 - Increased
Fibrinogen - Increased
Cholesterol - 2 fold increase
Triglycerides - Increased

Viral hepatitis in pregnancy is viewed with great alarm because of greater risks of fulminant hepatic failure setting in particularly in the last trimester, intrapartum and immediate postpartum period. Such events are definitely greater in pregnancy compared to men and non-pregnant women. Further pregnancy understandably being a Two (or even three) in one state, the problem is not only to mother but also pertains to the offspring. This includes foetal loss, mostly premature labour and vertical transmission of illness to the newborn especially in HBV infection.95,164

Vertical transmission of HEV

HEV infection has increased incidence in pregnant women and during epidemics icteric disease occurs 9 times more often in pregnant women than in men and non-pregnant women.96

HEV is commonly transmitted from infected mothers to their babies with significant perinatal morbidity and mortality. In a study conducted on eight pregnant women with viral hepatitis who were negative for markers of
HAV, HBV and HCV, HEV-RNA was detected in 5 mothers in their acute phase sera and anti-HEV IgM was positive in all the cases. The cord blood collected showed evidence of HEV-RNA by RT-PCR in 2 cases and infection by vertical transmission in 5 of the 8 infants (HEV-RNA). All these infants had anti-HEV IgG in their birth samples, thus confirming intrauterine transmission of HEV. Of the 8 cases, 4 (50%) passed onto fulminant hepatic failure. However, no chronic sequelae has been reported as a result of vertical transmission of HEV.

Although foetal and perinatal mortality was very high in patients with fulminant hepatic failure, even pregnant women with non-fulminant / uncomplicated hepatitis E had a higher (12.4%) rate of abortion and intrauterine death.

HEV preferentially affects women in the third trimester of pregnancy seemingly because of significant humoral immune suppression in these subjects, particularly so because the native acquired herd immunity to the infection is low.

**Molecular epidemiology of HEV**

To date the genomes of four geographically distinct strains of HEV have been cloned and completely sequenced which include Burma, Mexico, Pakistan and China isolates. Molecular sequencing diverges between isolates from Asia (Burma) and North America (Mexico) in the ORF 1 region. When the
Chinese HEV sequence was compared with the prototype Burmese HEV sequence, the homology between the two isolates was 93.2% and 98.5% at the nucleotide and aminoacid levels respectively. Nucleotide substitutions appeared to be distributed randomly throughout the genome. Reports from various studies suggest that the fragment of ORF1 between nucleotides 2011 and 2325 may be considered as the hypervariable region of the HEV genome. The region between nucleotides 6627 and 6603 of ORF 3 encoding for the putative HEV capsid protein exhibits a high degree of sequence conservation in several isolates of HEV.111

Recent studies from India on the ORF 3 region of HEV obtained from an outbreak in Kanpur, 1991, suggest that a large deletion (246 bp) in the amplified sequence exist when compared with the HEV Burma strain.169 However, nucleotide sequence analysis of a 2.2 kb portion of the HEV genome of the Indian strain obtained from the Hyderabad epidemic revealed a homology of 96.8% to the Burmese strain and did not observe the major deletion in the ORF 3.170