REVIEW

Hepatitis E: An overview and recent advances in clinical and laboratory research

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Abstract Hepatitis E virus (HEV) is a non-enveloped RNA (7.5 kb) virus that is responsible for large epidemics of acute hepatitis and a proportion of sporadic hepatitis cases in southeast and central Asia, the Middle East, parts of Africa and Mexico. Hepatitis E virus infection spreads by the faecal–oral route (usually through contaminated water) and presents after an incubation period of 8–10 weeks with a clinical illness resembling other forms of acute viral hepatitis. Clinical attack rates are the highest among young adults. Asymptomatic and anicteric infections are known to occur. Chronic HEV infection is not observed. Although the mortality rate is usually low (0.07–0.6%), the illness may be particularly severe among pregnant women, with mortality rates reaching as high as 25%. Recent isolation of a swine virus resembling human HEV has opened the possibility of zoonotic HEV infection. Studies of pathogenetic events in humans and experimental animals reveal that viral excretion begins approximately 1 week prior to the onset of illness and persists for nearly 2 weks; viraemia can be detected during the late phase of the incubation period. Immunoglobulin M antibody to HEV (anti-HEV) appears early during clinical illness but disappears rapidly over a few months. Immunoglobulin G anti-HEV appears a few days later and persists for at least a few years. There is no specific treatment available for hepatitis E virus infection. Ensuring a clean drinking water supply remains the best preventive strategy. Recombinant vaccines are being developed that may be particularly useful for travellers to disease-endemic areas and for pregnant women.
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Key words: enterically-transmitted virus, hepatitis E virus.

Hepatitis E, previously known as enterically transmitted non-A, non-B hepatitis, is an infectious viral disease with clinical and morphological features of acute hepatitis. The disease was first recognized as a distinct, clinical entity in the 1980s when sera from persons affected during a large waterborne epidemic of viral hepatitis during 1955–56 in Delhi, India[1] and another epidemic in Kashmir were found to lack serological markers of acute hepatitis A and B.[2,3] The occurrence of the first recorded epidemic of hepatitis E as late as 1955 and the infrequency of this disease in developed countries suggest that hepatitis E is a new, emerging infectious disease. However, several epidemics of enterically transmitted hepatitis with epidemiological features similar to those of hepatitis E outbreaks occurred in Europe and the United States in the 18th and 19th centuries.[4,5] It can be postulated, therefore, that hepatitis E virus (HEV) infection may have once been prevalent in various parts of the world and has only recently become restricted to certain geographical areas, mostly underdeveloped regions with poor environmental sanitation.

The first proof of the existence of a new viral hepatitis agent was obtained in 1983, when virus-like particles were detected by immune electron microscopy in faeces collected from a volunteer who was infected with faecal material from patients with suspected enterically transmitted non-A, non-B hepatitis.[6] The disease was successfully transmitted to cynomolgus monkeys who excreted similar virus-like particles in their faeces.[6] The

genome of this virus, now known as HEV,[7] was cloned in 1990[8] and fully sequenced shortly thereafter.[9,10]

VIROLOGY

Hepatitis E virus virions are small, non-enveloped, 32–34 nm diameter particles with icosahedral symmetry. The viral genome, approximately 7.5 kb in length, is a single-stranded, positive-sense, polyadenylated RNA molecule that contains short 5′ and 3′ non-coding regions of 27 and 68 nucleotides, respectively. It contains three overlapping open reading frames (ORF) (Fig. 1).[9] Open reading frame 1 is 5079 nt long and is predicted to code for a 1693 amino acid (aa) polyprotein consisting of non-structural proteins that are involved in viral genome replication and viral protein processing, as its sequence contains motifs characteristic of viral methyltransferases, papain-like cysteine proteases, helicases and RNA-dependent RNA polymerases. In addition, ORF1 has two regions called Y and X domains, whose functions remain unknown. The ORF2 begins 38 nt 3′ of the termination of ORF1 and consists of 1980 nt. It contains a typical signal sequence at its 5′ end, contains three probable glycosylation sites and encodes for the viral capsid protein. This protein is apparently synthesized in the endoplasmic reticulum as a 71-kDa, 660-aa long precursor, processed to a mature, glycosylated form (gpORF2), and then transported to the cell surface, directly or through the *cis*-Golgi compartment.[11] The mature form of ORF2 protein appears to arise by N-terminus truncation of the 660-aa ORF2 protein by 111 aa to a 58-kDa protein; the latter is processed further by a C-terminus truncation to produce a final 50-kDa protein, which has been shown to assemble into empty virus-like particles *in vitro*.[12,13] Open reading frame 3 is 369 nt long, overlaps ORF1 and ORF2 by 1 and 328 nt, respectively, and encodes for a 123-aa protein (pORF3) which is expressed intracellularly. Recent studies of the biology of HEV replication have shown that pORF3 may be capable of associating with the liver cell cytoskeleton.[14] The ORF3 protein also has a cysteine-rich region near its amino terminus and has been shown to bind HEV-RNA and enters into a complex with pORF2, the major capsid protein.[14] The pORF3 appears to serve as a cytoskeletal anchor site, where pORF2 and RNA can bind to begin the process of viral nucleocapsid assembly.[14] Several aspects of HEV genomic transcription, viral protein synthesis, final assembly and release of progeny virions remain unknown.

Establishment of a reliable *in vitro* tissue culture system for propagation of HEV will be critical for better understanding HEV replication. *In vivo*-infected hepatocytes have recently been shown to support HEV replication after isolation and placement into tissue culture.[15]

On the basis of its structural and physiochemical properties, HEV has provisionally been classified in the family *Caliciviridae*, genus *Calicivirus*.[16] However, its genomic organization more closely resembles that of rubella virus and plant furoviruses than that of caliciviruses.[17]

The HEV-RNA sequence data show that the virus has two main geographically distinct strains, Burmese (or Asian) and Mexican. Overall nucleotide homology among various isolates from Asia (e.g. India, Burma, Pakistan) ranges from 92 to 99% and that between Mexican and Burmese strains is much lower, being 75%.[10,18–20] The corresponding figures of variability in amino acid sequences are from 95 to 99%, and 86%, respectively. Hepatitis E virus isolates from Africa (Chad, Algeria) appear to be related more closely to the Burmese strain than to the Mexican strain.[21] Despite the genomic variability, the two geographically distinct isolates share at least one major cross-reactive epitope, as evidenced by data from various serological tests identifying anti-HEV antibodies.

A new isolate of HEV was recently identified in the United States in a patient who had no history of travel to the disease-endemic areas. Molecular analysis
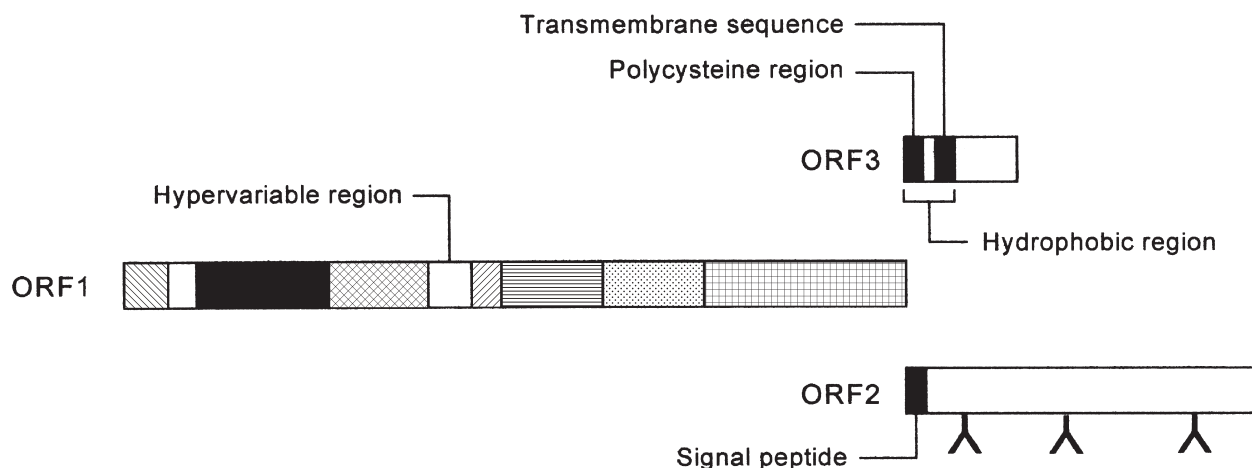
Figure 1   Genomic organisation of hepatitis E virus. ORF, open reading frame. (▨) Methyl transferase, (■) domain Y, (▧) papain-like cysteine protease, (▨) proline 'hinge', (▤) domain X, (⊡) helicase, (▥) RNA replicase, (人) potential glycosylation site.

of this isolate, designated HEV US-1, has shown only 78.2–79.6% nucleic acid identity and 90.2–91.8% amino acid homology in an ORF2 region of the Mexican and the Burmese HEV strains, respectively.[22,23] The serum of the infected patient was positive for immunoglobulin (Ig)G anti-HEV antibody, indicating immunological cross-reactivity with the structural protein of the other HEV strains. The HEV US-1 is genetically similar to swine HEV recently identified in pigs in the midwestern United States.[24] These two viruses have 97% homology in ORF2 at the amino acid level, and 92.1 and 93.5% homology in ORF3 at nucleic acid and amino acid levels, respectively.[24] Swine HEV, which appears to be ubiquitous in pigs, naturally infects most pigs before 3 months of age, inducing transient viraemia and histological hepatitis without clinical illness.[24] Antibody to swine HEV cross-reacts with antibody to the capsid protein of human HEV. A comparison of swine HEV with Burmese and Mexican isolates of human HEV has shown approximately 90.2–91.7% and 78.9–82.9% identity at the amino acid level in ORF2 and ORF3 regions, respectively.[22] In comparison, the US isolate of HEV had 97.7 and 93.5% identity with swine HEV at the aa level in these regions, respectively, indicating that the US isolate is phylogenetically closer to swine HEV.[22] In recent experimental studies, the US strain of human HEV has been shown to be infectious to specific, pathogen-free pigs and swine HEV has been transmitted to primates, suggesting that swine HEV may be capable of infecting humans.[25] However, in another study, Asian and Mexican isolates of HEV failed to induce disease in pigs.[26] Thus, further studies are required to elucidate the relationship of swine HEV to human HEV.

EPIDEMIOLOGY

Main epidemiological features

Hepatitis E virus infection is endemic in Southeast and Central Asia. Several outbreaks of hepatitis E have been observed in the Middle East, northern and western parts of Africa and North America (Mexico)[27] (Fig. 2). In other parts of the world, HEV infection is infrequent and is restricted predominantly to persons who have travelled to disease-endemic areas.

Hepatitis E outbreaks are large, affect several hundred to several thousand persons, and vary from short-lived, single-peaked outbreaks to prolonged, multimodal epidemics lasting for more than a year (Table 1).[1,3,28,29] During these outbreaks, overall attack rates range from 1 to 15%, being higher among adults (3 to 30%) than among children (0.2 to 10%).[3,28,30,31] The lower attack rates among children may reflect a higher frequency of anicteric and/or subclinical HEV infections in this age group. Males are usually more frequently affected. The outbreaks are characterized by a particularly high attack rate and mortality (as high as 25%) among pregnant women.[1,3,28,29] In areas where hepatitis E outbreaks occur, HEV infection accounts for a substantial proportion of acute sporadic hepatitis in both children and adults. In India, HEV infection accounts for 50 to 70% of all patients with sporadic viral hepatitis.[32,33] Demographic and clinical features of patients with sporadic hepatitis E (age distribution of cases, severity and duration of illness, worse prognosis among pregnant women and absence of chronic sequelae) resemble those of patients with epidemic hepatitis E.[34]
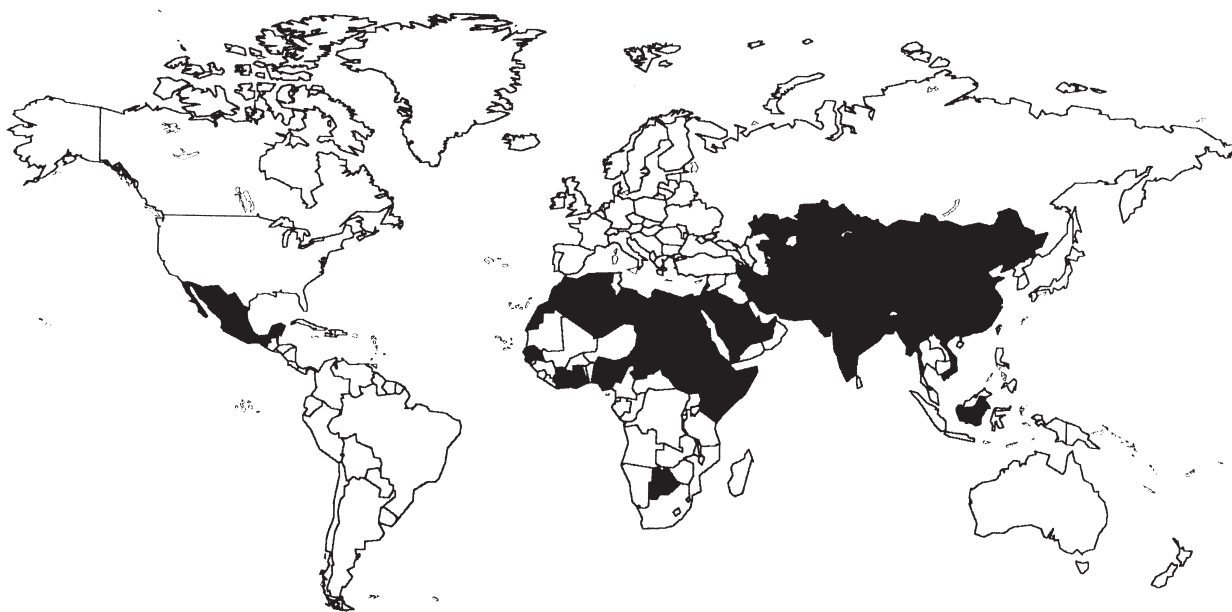
Figure 2 Geographical distribution of hepatitis E virus endemicity. The shaded areas represent disease-endemic regions.

Table 1 Epidemiological features of hepatitis E

Large outbreaks involving several thousand persons in
    developing countries
Sporadic hepatitis cases frequent in disease-endemic areas
Sporadic hepatitis cases uncommon in non-endemic areas
    (occur mainly among travellers to disease-endemic areas)
Faecal–oral transmission (usually through contaminated
    water)
Highest attack rate among young adults aged 15–40 years,
    with relative sparing of children
Insignificant person-to-person transmission
No evidence of parenteral or sexual transmission
Mother-to-newborn (transplacental) transmission probable
High attack rate among pregnant women, particularly those
    in second and third trimesters

Sporadic hepatitis E has been observed in several countries where outbreaks have not been reported, including Egypt, Hong Kong, Senegal and Turkey.[35–39] In non-endemic regions, the disease accounts for fewer than 1% of reported cases of acute viral hepatitis; these hepatitis E cases are almost always associated with travel to HEV-endemic regions,[40,41] although some cases have been reported among persons with no history of such travel.[42–46] In the United States, all cases of HEV infection had been related to travel to disease-endemic countries until recently, when HEV infection was reported in a patient without such a travel history.[23] Molecular studies have shown that the patient was infected with the novel HEV isolate (HEV US-1) described above.

Transmission, routes of spread and reservoirs

The faecal–oral route is the predominant mode of transmission of epidemic HEV infection. Most reported epidemics have been shown to be related to consumption of faecally contaminated drinking water.[1,3,28,31] The outbreaks frequently follow heavy rains and floods, when water sources become contaminated.[1,31,47] Some epidemics have occurred in hot summer months, when the reduction of water flow in rivers and streams may contribute to an increased risk of infection.[28,48] In the Indian subcontinent, China, Indonesia and Central Asian republics of the former Soviet Union, a pattern of recurrent epidemics has been observed; this is probably related to continuous existence of conditions that allow faecal contamination of water.

During hepatitis E outbreaks, person-to-person transmission of HEV appears to be distinctly uncommon.[1,49,50] Secondary attack rates among household members of hepatitis E cases are only 0.7–2.2%; in contrast, 50–75% of susceptible household contacts of hepatitis A cases are known to become infected.[51,52] Even when multiple cases occur among members of a family, such occurrence is related to exposure to a common source of contaminated water rather than to

person-to-person spread.[49] The mode of transmission responsible for sporadic HEV infections is unclear. Water contamination appears to be responsible for most cases in this setting; it is, however, plausible that food and fomites, and even person-to-person spread, may play a role. Nosocomial spread of HEV was presumed to be responsible for acute hepatitis in three health-care workers in South Africa who had treated a patient with fulminant hepatic failure due to HEV infection.[53] The reasons for differences in transmission patterns of hepatitis A virus and HEV are presently unclear but may relate to differences in viral titres in stools of infected persons, number of viral particles required to cause disease, or viability of these two viruses in the environment.

Vertical transmission of HEV infection from mother to infant is known to occur. In one study, six of eight babies born to mothers who had either acute uncomplicated hepatitis or fulminant hepatic failure from HEV infection in the third trimester of pregnancy were found to have evidence of HEV infection.[54] Of these six babies, five had HEV-RNA in their cord blood; another one baby had serological evidence of HEV infection acquired before birth. There is no evidence for sexual transmission of HEV, although in a report from Italy, 20% of homosexual men had anti-HEV antibodies as compared with only 3% of intravenous drug users,[55] thus suggesting faecal–oral transmission of HEV. There is no evidence for transmission of HEV by transfusion of blood or blood products; anti-HEV antibody prevalence rates among patients with haemophilia and thalassaemia and intravenous drug users are similar to those for the general population.[56,57]

Presumably, an environmental reservoir of HEV exists in disease-endemic areas that is responsible for recurrent epidemics. Laboratory investigations have shown, however, that HEV is a labile virus when exposed to high concentrations of salt, freeze–thawing and pelleting.[58] Another potential reservoir for persistence of HEV during interepidemic periods in disease-endemic areas may be in the form of serial transmission among susceptible individuals who have sporadic or subclinical hepatitis E. Recent data suggest that hepatitis E may be a zoonotic disease. Hepatitis E virus-RNA has been detected in the faeces of domestic swine in Nepal[59] and anti-HEV antibodies have been detected in the sera of pigs, cattle, sheep and rodents in disease-endemic areas.[59,60] Piglets and lambs have been shown to develop transaminasaemia, histological changes in the liver and viral excretion in faeces after experimental HEV infection.[61,62] In addition, as referred to above, a US isolate of HEV and swine HEV have been shown to be phylogenetically related[22] and to possess cross-species infectivity,[26] lending further support to the zoonosis hypothesis.

DIAGNOSIS AND SEROPREVALENCE DATA

Current laboratory tests for diagnosis of human HEV infection include molecular and immune electron

microscopy methodologies for detection of the virus in stool or serum and serological assays for identification of anti-HEV antibody of IgM and/or IgG class.

Genomic sequences of HEV can be detected in serum and stool specimens by reverse transcription–polymerase chain reaction (RT-PCR).[63] A recent modification of RT-PCR for HEV-RNA from serum and stool has provided a 1–2-log improvement in sensitivity of this technique and made it more reproducible.[64] Detection of HEV particles in stool specimens by immune electron microscopy is a cumbersome and insensitive technique presently almost never used in diagnostic laboratories.[6,65–67] The HEV antigen was detected in liver tissue only in experimental pathogenetic studies in primates.[68]

Serological diagnosis of HEV infection most commonly uses enzyme immunoassays (EIA).[69–73] Target antigens in those assays are either recombinant HEV proteins or synthetic HEV peptides that correspond to immunodominant epitopes of structural HEV proteins (ORF2 and ORF3) of the two main HEV strains derived from Burma and Mexico. More recently, a synthetic gene encoding several linear immunodominant antigenic epitopes from ORF2 and ORF3 regions has been synthesized and expressed as a mosaic protein that has been used in a solid-phase EIA.[74]

During acute HEV infection, IgM anti-HEV precedes the IgG by a few days, appears during early phase of clinical illness and disappears rapidly over 4–5 months.[69] In one study of sera collected from patients during various hepatitis E outbreaks 1–40 days, 3–4 and 6–12 months after the onset of jaundice, 100, 50 and 40%, respectively, tested positive for IgM anti-HEV.[74] In outbreak settings, IgM anti-HEV has been detected in more than 90% of patient serum samples obtained within 1 week to 2 months after the onset of illness. The IgG response appears shortly after the IgM response, and its titre increases throughout the acute phase into the convalescent phase, remaining high from 1 to 4.5 years after the acute phase of illness.[69,72] The exact duration of persistence of anti-HEV is not known. In one study, anti-HEV was detected in 47% of persons 14 years after acute HEV infection.[75] Determination of IgM anti-HEV is useful for the diagnosis of acute infection, whereas the presence of IgG anti-HEV indicates HEV infection, not necessarily recent.

The prevalence of anti-HEV in healthy subjects has been studied in various populations worldwide to measure the extent of exposure to HEV. It has been found that anti-HEV antibodies are present in persons living in all geographical areas. In disease-endemic areas of Asia and Africa, the prevalence rates among healthy populations are much higher than those in non-endemic areas. In most disease-endemic areas, anti-HEV has been detected in as many as 5% of children less than 10 years of age, and this ratio increases to 10–40% among adults older than 25 years of age.[37,76–79] These findings suggest that HEV infection, unlike that of other enterically transmitted agents, is infrequent among young children in developing countries. However, in a recent report from India, anti-HEV antibodies were detected in more than 60% of children below the age of 5 years.[80] These differences between

different disease-endemic areas may be related to varying epidemiological conditions in different geographical areas, differences in diagnostic techniques used, or both.

In developed countries of Europe and North America, 1–5% of the population have anti-HEV;[44,72,79,81,82] this range appears to be relatively high compared with the low rate of clinically evident hepatitis E disease in these areas. However, in a recent study that used two different serological tests to estimate the prevalence of anti-HEV, concordance between the two tests was only 27%.[83] Thus, it remains unclear whether the anti-HEV seroreactivity in non-endemic areas reflects subclinical and/or anicteric HEV infection, serological cross-reactivity with other agents, false-positivity of serological tests, or a combination of all these factors. It is also conceivable that relatively high prevalence of anti-HEV among healthy individuals in the United States may be related to subclinical infection with swine HEV.

Enzyme immunoassays currently used in various laboratories were directly compared using a panel of coded serum samples.[84] This study showed that their sensitivity rates varied widely (from 17 to 100%), and concordance rates among reactive serum specimens ranged from none to 89% (median 32%).[84] The assays compared in this study used recombinant HEV proteins or synthetic peptides, which differ in length, part of the genome with which they correlate, and the geographical strain of HEV to which they correspond. The study suggested that currently available assays for detection of anti-HEV are proficient when applied to patients with acute disease, particularly in disease-endemic areas, whereas the interpretation of seroprevalence studies that use anti-HEV assays remains questionable.

CLINICAL FEATURES

The incubation period of hepatitis E ranges from 2 to 10 weeks.[1,6,85,86] Clinical manifestations of HEV infection are similar to those of infection with other hepatitis viruses and encompass a wide spectrum of symptoms (Table 2).

Acute icteric hepatitis, the commonest recognizable form of illness, is usually insidious in onset and has an initial prodromal phase lasting a few days, with a variable combination of flu-like symptoms, fever, mild chills, abdominal pain, anorexia, nausea, aversion to smoking, vomiting, clay-coloured stools, dark or tea-coloured urine, diarrhoea, arthralgia, asthenia and a transient macular skin rash (Table 3).[3,87–90] These symptoms are followed in a few days by darkening of the urine, lightening of the stool colour and the appearance of jaundice. Itching may also occur. With the onset of jaundice, fever and other prodromal symptoms tend to diminish rapidly and soon disappear entirely.

Physical examination reveals jaundice and a mildly enlarged, soft and slightly tender liver and, in some patients, a soft, palpable spleen.[88] Laboratory test abnormalities include bilirubinuria, variable degree of rise in serum bilirubin (predominantly conjugated),

marked elevation in serum alanine aminotransferase (ALT), aspartate aminotransferase and gamma-glutamyltransferase activities, and a mild rise in serum alkaline phosphatase activity. A rise in aminotransferase levels may precede the onset of symptoms by as long as 10 days and reaches a peak by the end of the first week. The magnitude of transaminase rise does not correlate well with the severity of liver injury. As the illness subsides, serum aminotransferase and bilirubin abnormalities start receding, reaching normal values by 6 weeks in most patients.[88]

The illness is usually self-limiting and typically lasts 1–4 weeks.[87,88] No evidence of chronic hepatitis or cirrhosis has been detected following acute hepatitis E.[91–93] A few patients, however, have a prolonged clinical illness with marked cholestasis (cholestatic hepatitis), including persistent jaundice and prominent itching. In these cases, laboratory tests show a rise in alkaline phosphatase and a persistent bilirubin rise even after transaminase levels have returned to normal. The prognosis is good as jaundice finally resolves spontaneously after 2–6 months.

Other infected individuals have a milder clinical course and develop only non-specific symptoms that resemble those of an acute viral febrile illness without jaundice (anicteric hepatitis). In these patients, liver involvement is recognized only if laboratory studies are performed. In its most benign form, HEV infection is entirely unapparent and asymptomatic and passes unnoticed. The exact frequencies of asymptomatic infection and of anicteric hepatitis are not known but probably far exceed that of icteric disease as, in disease-endemic areas, a large proportion of individuals who test positive for anti-HEV antibodies do not recall having had jaundice.

A small proportion of patients have a more severe disease with fulminant or subacute (or late-onset) hepatic failure. In disease-endemic regions, this infection constitutes an important cause of fulminant hepatic failure. In India, for instance, HEV infection (alone or in combination with other hepatitis viruses) was responsible for 62% of adult patients and 40% of children with sporadic fulminant hepatitis.[94,95] In Pakistan, two-thirds of a group of pregnant women with fulminant liver failure had HEV infection.[96]

The case-fatality rate in many reports has ranged from 0.5 to 4%;[50,97] these reports, however, are based on hospital data and, thus, may over-estimate mortality. Studies based on population surveys during outbreaks report lower mortality rates varying from 0.07 to 0.6%.[28,87,97] In an epidemic among army personnel in Ethiopia, no patient among 423 individuals with icteric hepatitis developed fulminant hepatic failure or died.

Pregnant women, particularly those in the second and third trimesters, are more frequently affected during hepatitis E outbreaks and have a worse outcome. Mortality rates among pregnant women, especially those infected in the third trimester, range between 15 and 25%.[30,31,50,98] In an epidemic in Kashmir, India, attack rates among those in the first, second and third trimesters were 8.8, 19.4 and 18.6%, respectively, as compared with 2.1% among non-pregnant women and 2.8% among men.[98] Further, fulminant hepatic failure developed in 22.2% of the affected pregnant women, in comparison with 2.8 and 0% of affected men and non-pregnant women, respectively. Frequency of abortions, still births and neonatal deaths is also increased among pregnant women with HEV infection.[30]

Histological features of hepatitis E may differ from those of other forms of acute viral hepatitis. Nearly half of hepatitis E patients have a cholestatic-type of hepatitis, which is characterized by canalicular bile stasis and gland-like transformation of parenchymal cells. In these patients, degenerative changes in hepatocytes are less

Table 2 Clinical features of hepatitis E

Incubation period 2–10 weeks
Variable clinical manifestations including:
Icteric hepatitis
Severe hepatitis leading to fulminant hepatic failure
Anicteric hepatitis
Inapparent, asymptomatic infection
Clinical illness similar to other viral hepatitides (except among pregnant women)
Milder illness in children
Low mortality rate (0.07–0.6%)
High mortality (15–25%) among pregnant women
No relationship with chronic hepatitis, cirrhosis or hepatocellular carcinoma

Table 3 Clinical findings (% occurrence) in hepatitis E outbreaks

| Symptoms and signs	Delhi, India, 1956 ($n=958$)	Accra, Ghana, 1963 ($n=136$)	Kashmir, India, 1978 ($n=275$)	Ethiopia, 1989 (%) ($n=423$)	Xinjing, China, 1986–88 ($n=85$)
Jaundice	100	100	100	100	91
Malaise		95		100	95
Anorexia	66	95	79	100	69
Abdominal pain	63	37	41	82	55
Hepatomegaly	62	67	85	10	80
Nausea, vomiting	29	48	46	100	91
Fever	23	57	44	97	53
Pruritus	14	47	20	14	59

marked.[91] In other patients, changes resemble those of other forms of acute hepatitis, such as the presence of ballooned hepatocytes and acidophilic bodies, and focal or confluent hepatocyte necrosis. No particular zonal distribution of hepatocyte damage is observed. In both forms, lobules contain an inflammatory infiltrate consisting predominantly of macrophages and lymphocytes and, in patients with a cholestatic type of hepatitis, of a few polymorphonuclear leucocytes. The Kupffer cells appear prominent. Portal tracts are enlarged and contain an inflammatory infiltrate consisting of lymphocytes and a few polymorphonuclear leucocytes and eosinophils; polymorphonuclear cells are particularly increased in the cholestatic type of lesion.[91] In cases with severe liver injury, a large proportion of hepatocytes are affected, leading to submassive or massive necrosis with collapse of liver parenchyma.

PATHOGENESIS

Several elements of pathogenesis can be outlined on the basis of data from human patients and those from experimentally infected animals (Fig. 3). The incubation period in human volunteers after oral exposure is 4–5 weeks,[6,86] but the route and mechanism by which the virus reaches the liver from the intestinal tract remain unknown. Hepatitis E virus can be detected in stools beginning approximately 1 week before the onset of illness and persists for as long as 2 weeks thereafter.[6,29,98–100] Hepatitis E virus-RNA can be detected in faeces of most patients with acute hepatitis E by RT-PCR for approximately 2 weeks;[101,102] in some cases, RT-PCR has yielded positive results for as long as 52 days after onset.[103] The HEV-RNA has regularly been found in serum by RT-PCR in virtually all patients in the first 2 weeks after the onset of illness;[101] prolonged periods of HEV-RNA positivity in serum ranging from 4 to 16 weeks have also been reported.[86,103]

Experimental infection with HEV leading to varying levels of virus excretion, liver enzyme elevations and histopathological changes in liver has been demonstrated in several non-human primates, including cynomolgus macaques, chimpanzees,[100] rhesus[104,105] and owl monkeys,[106] and tamarins.[107] Hepatitis E virus infection in cynomolgus macaques is a reproducible and widely used experimental model that has provided valuable data regarding pathogenetic events in this infection.[68,100,106–110] Infection can be transmitted to cynomolgus macaques by either the intravenous or oral route; the former, however, is much more reproducible. In this model, the average incubation period for acute hepatitis E is approximately 21 days. Hepatitis E virus-
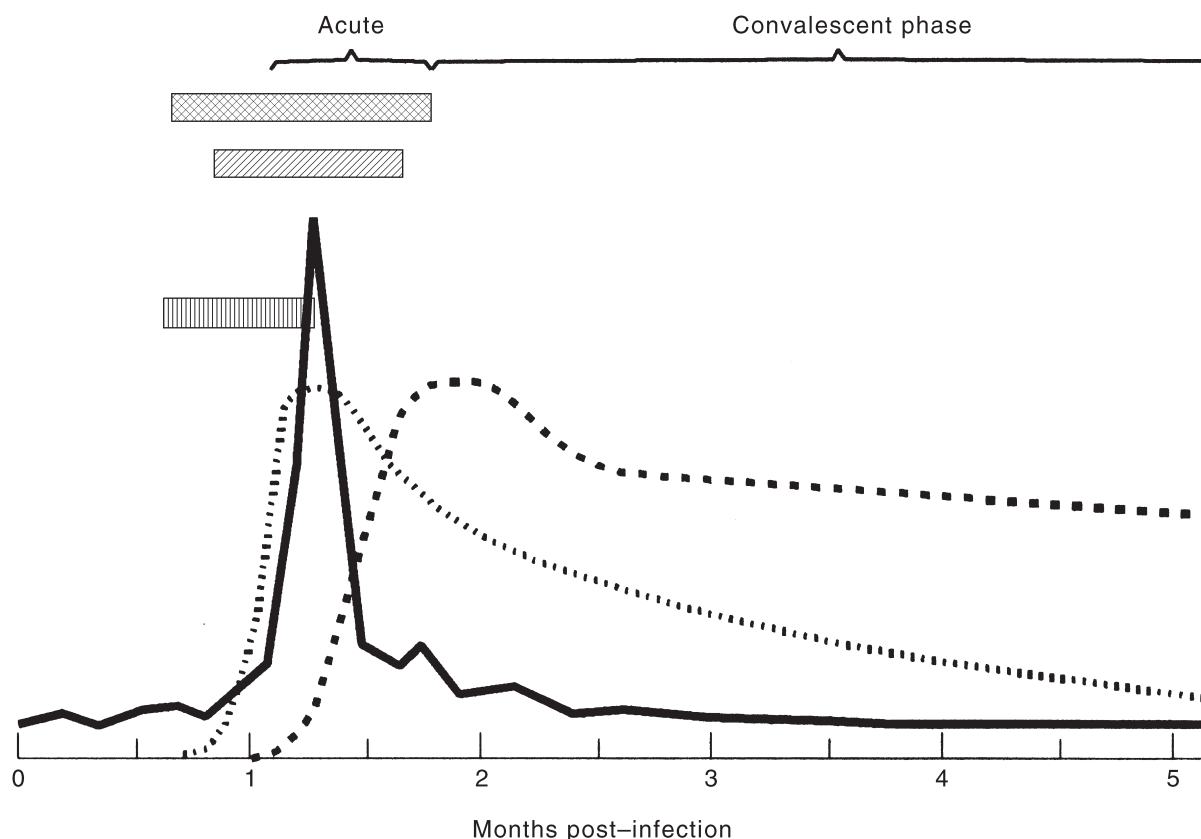
Figure 3 A graphical representation showing time course of events during hepatitis E virus (HEV) infection based on studies in human subjects and in experimentally infected primates. (—) alanine aminostransferase, (– –) immunoglobulin (Ig) G antibody to HEV (anti-HEV), (- - -) IgM anti-HEV, (⊠) HEV-RNA in stool, (⊘) HEV antigen in the liver, (⊞) HEV-RNA in serum.

RNA, as detected by RT-PCR, appears in serum, bile and faeces a few days before the onset of the transaminase rise. After intravenous inoculation of HEV in cynomolgus macaques, expression of HEV antigen in hepatocytes, indicative of viral replication, first appears at approximately day 7 post-infection. It reaches a peak, at which it can be detected in 70–90% of hepatocytes and begins to decline after the ALT peak is reached (K Krawczynski *et al.* unpubl. data, 1989). Hepatitis E virus antigen has been detected simultaneously in hepatocyte cytoplasm, bile and faeces during the second or third week after inoculation, and before and concurrently with the onset of ALT elevation and histopathological changes in the liver.[106,108,109] These findings suggest that HEV may be released from hepatocytes into bile before the peak of morphological changes in the liver, during the highly replicative initial phase of infection. The onset of ALT elevation and the presence of histopathological changes in the liver generally correspond to the detection of anti-HEV in serum and with decreasing levels of HEV antigen in hepatocytes. These findings suggest that liver injury may be largely immune-mediated, especially as infiltrating lymphocytes in the liver have been found to have a cytotoxic/suppressor immunophenotype.[110] The reason for particularly severe liver damage in pregnant women with hepatitis E is not known.

PREVENTION

Preventing hepatitis E in disease-endemic areas depends primarily on providing a clean drinking water supply and strict attention to sewage disposal. During an epidemic, steps to improve water quality can lead to rapid abatement of the occurrence of new cases.[47] Boiling water before consumption appears to reduce the risk of acute hepatitis E.[111] Isolation of affected persons is not indicated as person-to-person transmission is uncommon.[49]

The protective role of anti-HEV antibodies in humans requires further study. The occurrence of large hepatitis E epidemics among adults in disease-endemic areas suggests either that anti-HEV antibody may not be fully protective or that antibody levels decline with time and gradually reach an unprotective level. However, no reduction in disease rates could be shown in pre- or post-exposure prophylaxis studies among recipients of immunoglobulin preparations manufactured in hepatitis E-endemic areas.[112,113] In a recent study, administration of immune serum globulin to pregnant women during an outbreak was shown to reduce the number of total fresh HEV infections, although the number of clinical cases was unchanged.[114] However, in another study, intramuscular administration of serum obtained from a human volunteer 4 years after acute HEV infection did not confer any protection against HEV challenge to monkeys.[115] Thus, further work may be necessary before a definitive opinion about the role of immune serum globulin can be made.

In experimental studies in primates, passive transfer of anti-HEV has been shown to alleviate the course of HEV infection.[116] Susceptible primates (cynomolgus macaques) were also used in preliminary trials of HEV recombinant vaccines.[117,118] The earlier studies showed protection against hepatitis and viraemia following HEV challenge after immunization with recombinant proteins corresponding to HEV capsid protein, but faecal excretion of the virus was not prevented.[119] More recently, immunization of mice with naked complementary DNA corresponding to HEV capsid protein was shown to induce the development of anti-HEV.[120] This latter approach, if successful, may be advantageous as DNA immunization usually induces cellular immune response in addition to antibody response and, thus, might provide a longer duration of protection. In addition, swine HEV, which has immunological cross-reactivity with antibodies against the capsid protein of human HEV, may be considered for experimental vaccination to induce anti-HEV antibodies protective against subsequent HEV challenge. More studies in susceptible primates are needed to evaluate the experimental HEV vaccines, as even short-term protection conferred by a vaccine may be useful for travellers to disease-endemic areas and for persons at high risk of developing a serious illness if infected, such as pregnant women living in HEV-endemic areas.

ACKNOWLEDGEMENTS

REFERENCES

1 Vishwanathan R. Infectious hepatitis in Delhi (1995–96): A critical study: Epidemiology. *Indian J. Med. Res.* 1957; **45** (Suppl. 1): 1–29.

2 Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM. Epidemic and endemic hepatitis in India: Evidence for a non-A, non-B hepatitis etiology. *Lancet* 1980; **2**: 876–9.

3 Khuroo MS. Study of an epidemic of non-A, non-B hepatitis: Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am. J. Med.* 1980; **68**: 818–23.

4 Cockayne EA. Cattarhal jaundice, sporadic and epidemic, and its relation to acute yellow atrophy of the liver. *Q. J. Med.* 1912; **6**: 1.

5 Blumer GB. Infectious hepatitis in the United States. *JAMA* 1923; **49**: 230–4.

6 Balayan MS, Andjaparidze AG, Savinskaya SS *et al.* Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 1983; **20**: 23–31.

7 Purcell RH, Ticehurst JR. Enterically transmitted non-A, non-B hepatitis: Epidemiology and clinical characteristics. In: Zuckermann AJ, ed. *Viral Hepatitis and Liver Disease.* New York: Alan R Liss, 1988; 131–7.

8 Reyes GR, Purdy MA, Kim JP *et al.* Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 1990; **247**: 1335–9.

9 Tam AW, Smith MM, Guerra ME *et al.* Hepatitis E virus (HEV): Molecular cloning and sequencing of the full-length viral genome. *Virology* 1991; **185**: 120–31.

10 Tsarev SA, Emerson SU, Reyes GR *et al.* Characterization of a prototype strain of hepatitis E virus. *Proc. Natl Acad. Sci. USA* 1992; **89**: 559–63.

11 Jameel S, Zafrullah M, Ozdener MH, Panda SK. Expression in animal cells and characterization of the hepatitis E virus structural proteins. *J. Virol.* 1996; **70**: 207–16.

12 McAtee CP, Zhang Y, Yarbough PO, Bird T, Fuerst TR. Purification of a soluble hepatitis E open reading frame 2-derived protein with unique antigenic properties. *Protein Exp. Purif.* 1996; **8**: 262–70.

13 Li TC, Yamakawa Y, Suzuki K *et al.* Expression and self-assembly of empty virus-like particles of hepatitis E virus. *J. Virol.* 1997; **71**: 7207–13.

14 Zafrullah M, Ozdener MH, Panda SK, Jameel S. The ORF3 protein of hepatitis E virus is a phosphoprotein that associates with the cytoskeleton. *J. Virol.* 1997; **71**: 9045–53.

15 Tam AW, White R, Reed E *et al. In vitro* propagation and production of hepatitis E virus from *in vivo*-infected primary macaque hepatocytes. *Virology* 1996; **215**: 1–9.

16 Cubitt D, Bradley DW, Carter MJ *et al.* Caliciviridae. Virus Taxonomy. *Arch. Virol.* 1995; **10** (Suppl.): 359–63.

17 Koonin EV, Gorbalenya AE, Purdy MA, Rozanov MN, Reyes GR, Bradley DW. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: Delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc. Natl Acad. Sci. USA* 1992; **89**: 8259–63.

18 Yin S, Tsarev SA, Purcell RH, Emerson SU. Partial sequence comparison of eight new Chinese strains of hepatitis E virus suggests the genome sequence is relatively stable. *J. Med. Virol.* 1993; **41**: 230–41.

19 Bi SL, Purdy MA, McCaustland KA, Margolis HS, Bradley DW. The sequence of hepatitis E virus isolated directly from a single source during an outbreak in China. *Virus Res.* 1993; **28**: 233–47.

20 Huang CC, Nguyen D, Fernandez J, Yun KY, Fry KE, Bradley DW *et al.* Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). *Virology* 1992; **191**: 550–8.

21 van Cuyck-Gandre H, Zhang HY, Tsarev SA *et al.* Characterization of hepatitis E virus (HEV) from Algeria and Chad by partial genome sequence. *J. Med. Virol.* 1997; **53**: 340–7.

22 Schlauder GG, Dawson GJ, Erker JC *et al.* The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *J. Gen. Virol.* 1998; **79**: 447–56.

23 Kwo PY, Schlauder GG, Carpenter HA *et al.* Acute hepatitis E by a new isolate acquired in the United States. *Mayo Clin. Proc.* 1997; **72**: 1133–6.

24 Meng XJ, Purcell RH, Halbur PG *et al.* A novel virus in swine is closely related to the human hepatitis E virus. *Proc. Natl Acad. Sci. USA* 1997; **94**: 9860–5.

25 Meng XJ, Halbur PG, Shapiro MS *et al.* Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J. Virol.* 1998; **72**: 9714–21.

26 Meng XJ, Halbur PG, Haynes JS *et al.* Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strains of HEV. *Arch. Virol.* 1998; **143**: 1405–15.

27 Krawczynski K. Hepatitis E. *Hepatology* 1993; **17**: 932–41.

28 Naik SR, Aggarwal R, Salunke PN, Mehrotra NN. A large waterborne viral hepatitis E epidemic in Kanpur, India. *Bull. WHO* 1992; **70**: 597–604.

29 Zhuang H. Hepatitis E and strategies for its control. In: Wen YM, Xu ZY, Melnick JL, eds Viral hepatitis in China: Problems and Control Strategies. 1992; 126–39.

30 Tsega E, Hansson BG, Krawczynski K, Nordenfelt E. Acute sporadic viral hepatitis in Ethiopia: causes, risk factors, and effects on pregnancy. *Clin. Infect Dis* 1992; **14**: 961–5.

31 Kane MA, Bradley DW, Shrestha SM *et al.* Epidemic non-A, non-B hepatitis in Nepal: Recovery of a possible etiologic agent and transmission studies in marmosets. *JAMA* 1984; **252**: 3140–5.

32 Khuroo MS, Rustgi VK, Dawson GJ *et al.* Spectrum of hepatitis E virus infection in India. *J. Med. Virol.* 1994; **43**: 281–6.

33 Arankalle VA, Chobe LP, Jha J *et al.* Aetiology of acute sporadic non-A, non-B viral hepatitis in India. *J. Med. Virol.* 1993; **40**: 121–5.

34 Khuroo MS, Deurmeyer W, Zargar SA, Ahanger MA, Shah MA. Acute sporadic non-A, non-B, hepatitis in India. *Am. J. Epidemiol.* 1983; **118**: 360–4.

35 Coursaget P, Depril N, Yenen OS, Cavuslu S, Badur S. Hepatitis E virus infection in Turkey. *Lancet* 1993; **342**: 810–11.

36 el-Zimaity DM, Hyams KC, Imam IZ *et al.* Acute sporadic hepatitis E in an Egyptian pediatric population. *Am. J. Trop. Med. Hyg.* 1993; **48**: 372–6.

37 Lok AS, Kwan WK, Moeckli R *et al.* Seroepidemiological survey of hepatitis E in Hong Kong by recombinant-based enzyme immunoassays. *Lancet* 1992; **340**: 1205–8.

38 Goldsmith R, Yarbough PO, Reyes GR *et al.* Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet* 1992; **339**: 328–31.

39 Pillot J, Lazizi Y, Diallo Y, Leguenno B. Frequent sporadic hepatitis E in West Africa evidenced by characterization of a virus-associated antigen in the stool. *J. Hepatol.* 1992; **15**: 420–1.

40 Centers for Disease Control and Prevention. Hepatitis E among US travelers, 1989–92. *MMWR Morb. Mortal. Wkly Rep.* 1993; **42**: 1–4.

41 De Cock KM, Bradley DW, Sandford NL, Govindarajan S, Maynard JE, Redekar AG. Epidemic non-A, non-B hepatitis in patients from Pakistan. *Ann. Intern. Med.* 1987; **106**: 227–30.

42 Heath TC, Burrow JN, Currie BJ *et al.* Locally acquired hepatitis E in the Northern Territory of Australia. *Med. J. Aust.* 1995; **162**: 318–19.

43 Sallie R, Silva AE, Purdy M *et al.* Hepatitis C and E in non-A non-B fulminant hepatic failure: A polymerase chain reaction and serological study. *J. Hepatol.* 1994; **20**: 580–8.

44 Zanetti AR, Dawson GJ. Hepatitis type E in Italy: A sero-epidemiological survey. Study Group of Hepatitis E. *J. Med. Virol.* 1994; **42**: 318–20.

45 Tassopoulos NC, Krawczynski K, Hatzakis A *et al.* Case report: Role of hepatitis E virus in the etiology of com-

munity-acquired non-A, non-B hepatitis in Greece. *J. Med. Virol.* 1994; **42**: 124–8.

46 Chapman BA, Burt MJ, Wilkinson ID, Schousboe MI. Community acquired viral hepatitis in New Zealand: A case of sporadic hepatitis E virus infection. *Aust. NZ J. Med.* 1993; **23**: 722–3.

47 Bile K, Isse A, Mohamud O *et al.* Contrasting roles of rivers and wells as sources of drinking water on attack and fatality rates in a hepatitis E epidemic in Somalia. *Am. J. Trop. Med. Hyg.* 1994; **51**: 466–74.

48 Corwin A, Jarot K, Lubis I *et al.* Two years' investigation of epidemic hepatitis E virus transmission in West Kalimantan (Borneo), Indonesia. *Trans. Roy. Soc. Trop. Med. Hyg.* 1995; **89**: 262–5.

49 Aggarwal R, Naik SR. Hepatitis E intrafamilial transmission versus waterborne spread. *J. Hepatol.* 1994; **21**: 718–23.

50 Myint H, Soe MM, Khin T, Myint TM, Tin KM. A clinical and epidemiological study of an epidemic of non-A, non-B hepatitis in Rangoon. *Am. J. Trop. Med. Hyg.* 1985; **34**: 1183–9.

51 Villarejos VM, Serra J, Anderson-Visona K, Mosley JW. Hepatitis A virus infection in households. *Am. J. Epidemiol.* 1982; **115**: 577–86.

52 Greco D, De Giocomi G, Piersante GP, Bibby L, Nicastro M, Cavalcanti P. A person to person hepatitis A outbreak. *Int. J. Epidemiol.* 1986; **15**: 108–11.

53 Robson SC, Adams S, Brink N, Woodruff B, Bradley D. Hospital outbreak of hepatitis E. *Lancet* 1992; **339**: 1424–5.

54 Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet* 1995; **345**: 1025–6.

55 Montella F, Rezza G, Di Sora F, Pezzotti P, Recchia O. Association between hepatitis E virus and HIV infection in homosexual men. *Lancet* 1994; **344**: 1433.

56 Psichogiou M, Tzala E, Boletis J *et al.* Hepatitis E virus infection in individuals at high risk of transmission of non-A, non-B hepatitis and sexually transmitted diseases. *Scand. J. Infect. Dis.* 1996; **28**: 443–5.

57 Barzilai A, Schulman S, Karetnyi YV *et al.* Hepatitis E virus infection in hemophiliacs. *J. Med. Virol.* 1995; **46**: 153–6.

58 Bradley DW. Hepatitis E epidemiology, aetiology and molecular biology. *Rev. Med. Virol.* 1992; **2**: 19–28.

59 Clayson ET, Innis BL, Myint KS *et al.* Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. *Am. J. Trop. Med. Hyg.* 1995; **53**: 228–32.

60 Karetnyi IV, Dzhumalieva DI, Usmanov RK, Titova IP, Litvak II, Balaian MS. The possible involvement of rodents in the spread of viral hepatitis E. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 1993; **4**: 52–6.

61 Usmanov RK, Balaian MS, Kazachkov IA *et al.* Further study of experimental hepatitis E in piglets. *Vopr. Virusol.* 1994; **39**: 208–12.

62 Usmanov RK, Balaian MS, Dvoinikova OV *et al.* An experimental infection in lambs by the hepatitis E virus. *Vopr. Virusol.* 1994; **39**: 165–8.

63 McCaustland KA, Bi S, Purdy MA, Bradley DW. Application of two RNA extraction methods prior to amplification of hepatitis E virus nucleic acid by the polymerase chain reaction. *J. Virol. Methods* 1991; **35**: 331–42.

64 Aggarwal R, McCaustland KA. Hepatitis E virus RNA detection in serum and feces specimens with the use of microspin columns. *J. Virol. Methods* 1998; **74**: 209–13.

65 Ticehurst J. Identification and characterization of hepatitis E virus. In: Hollinger BF, Lemon SM, Margolis HS, eds. *Viral Hepatitis and Liver Disease*. Baltimore: Williams & Wilkins, 1991; 501–13.

66 Bradley DW, Krawczynski K, Cook EH *et al.* Enterically transmitted non-A, non-B hepatitis: Etiology of disease and laboratory studies in nonhuman primates. In: Zuckermann AJ, ed. *Viral Hepatitis and Liver Disease*. New York: Alan R Liss, 1988; 138–47.

67 Arankalle VA, Chadha MS, Mehendale SM, Banerjee K. Outbreak of enterically transmitted non-A, non-B hepatitis among school children. *Lancet* 1988; **ii**: 1199–200.

68 Krawczynski K, Bradley DW. Enterically transmitted non-A, non-B hepatitis: Identification of virus-associated antigen in experimentally infected cynomolgus macaques. *J. Infect. Dis.* 1989; **159**: 1042–9.

69 Favorov MO, Fields HA, Purdy MA *et al.* Serologic identification of hepatitis E virus infections in epidemic and endemic settings. *J. Med. Virol.* 1992; **36**: 246–50.

70 Li F, Zhuang H, Kolivas S, Locarnini SA, Anderson DA. Persistent and transient antibody responses to hepatitis E virus detected by western immunoblot using open reading frame 2 and 3 and glutathione S-transferase fusion proteins. *J. Clin. Microbiol.* 1994; **32**: 2060–6.

71 Tsarev SA, Tsareva TS, Emerson SU *et al.* ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: Identification of HEV infection in primates. *J. Infect. Dis.* 1993; **168**: 369–78.

72 Dawson GJ, Chau KH, Cabal CM, Yarbough PO, Reyes GR, Mushahwar IK. Solid-phase enzyme-linked immunosorbent assay for hepatitis E virus IgG and IgM antibodies utilizing recombinant antigens and synthetic peptides. *J. Virol. Methods* 1992; **38**: 175–86.

73 Coursaget P, Buisson Y, Depril N *et al.* Mapping of linear B cell epitopes on open reading frames 2- and 3-encoded proteins of hepatitis E virus using synthetic peptides. *FEMS Microbiol. Lett.* 1993; **109**: 251–5.

74 Favorov MO, Khudyakov YE, Mast EE *et al.* IgM and IgG antibodies to hepatitis E virus (HEV) detected by an enzyme immunoassay based on an HEV-specific artificial recombinant mosaic protein. *J. Med. Virol.* 1996; **50**: 50–8.

75 Khuroo MS, Kamili S, Dar MY, Moecklii R, Jameel S. Hepatitis E and long-term antibody status [letter]. *Lancet* 1993; **341**: 1355.

76 Arankalle VA, Tsarev SA, Chadha MS *et al.* Age-specific prevalence of antibodies to hepatitis A and E viruses in Pune, India, 1982 and 1992. *J. Infect. Dis.* 1995; 171: 447–50.

77 Thomas DL, Mahley RW, Badur S, Palaoglu KE, Quinn TC. Epidemiology of hepatitis E virus infection in Turkey. *Lancet* 1993; **341**: 1561–2.

78 Arif M, Qattan I, al-Faleh F, Ramia S. Epidemiology of hepatitis E virus (HEV) infection in Saudi Arabia. *Ann. Trop. Med. Parasitol.* 1994; **88**: 163–8.

79 Paul DA, Knigge MF, Ritter A *et al.* Determination of hepatitis E virus seroprevalence by using recombinant

fusion proteins and synthetic peptides. *J. Infect. Dis.* 1994; **169**: 801–6.

80 Aggarwal R, Shahi H, Naik S, Yachha SK, Naik SR. Evidence in favour of high infection rate with hepatitis E virus among young children in India. *J. Hepatol.* 1997; **26**: 1425–6.

81 Moaven L, Van Asten M, Crofts N, Locarnini SA. Sero-epidemiology of hepatitis E in selected Australian populations. *J. Med. Virol.* 1995; **45**: 326–30.

82 Karetnyi YV, Favorov MO, Khudyakova NS *et al.* Serological evidence for hepatitis E virus infection in Israel. *J. Med. Virol.* 1995; **45**: 316–20.

83 Mast EE, Kuramoto IK, Favorov MO *et al.* Prevalence of and risk factors for antibody to hepatitis E virus seroreactivity among blood donors in northern California. *J. Infect. Dis.* 1997; **176**: 34–40.

84 Mast EE, Alter MJ, Holland PV, Purcell RH. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology* 1998; **27**: 857–61.

85 Belabbes EH, Bouguermouh A, Benatallah A, Illoul G. Epidemic non-A, non-B viral hepatitis in Algeria: Strong evidence for its spreading by water. *J. Med. Virol.* 1985; **16**: 257–63.

86 Chauhan A, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK. Hepatitis E virus transmission to a volunteer. *Lancet* 1993; **341**: 149–50.

87 Vishwanathan R, Sidhu AS. Infectious hepatitis: Clinical findings. *Indian J. Med. Res.* 1957; **45** (Suppl.): 49–58.

88 Zhuang H, Cao X-Y, Liu C-B, Wang G-M. Enterically transmitted non-A, non-B hepatitis in China. In: Shikata T, Purcell RH, Uchida T, eds. *Viral Hepatitis C, D and E.* Amsterdam: Excerpta Medica, 1991; 277–85.

89 Morrow Jr RH, Smetana HF, Sai FT, Edgcomb JH. Unusual features of viral hepatitis in Accra, Ghana. *Ann. Intern. Med.* 1968; **68**: 1250–64.

90 Tsega E, Krawczynski K, Hansson BG *et al.* Outbreak of acute hepatitis E virus infection among military personnel in northern Ethiopia. *J. Med. Virol.* 1991; **34**: 232–6.

91 Gupta DN, Smetana HF. The histopathology of viral hepatitis as seen in the Delhi epidemic (1955–56). *Indian J. Med. Res.* 1957; **45** (Suppl.): 101–13.

92 Chadha MS, Arankalle VA, Banerjee K. Follow up of cases of enterically transmitted non-A, non-B hepatitis. *J. Assoc. Physicians India* 1991; **39**: 651–2.

93 Khuroo MS, Saleem M, Teli MR, Sofi MA. Failure to detect chronic liver disease after epidemic non-A, non-B hepatitis. *Lancet* 1980; **ii**: 97–8.

94 Nanda SK, Yalcinkaya K, Panigrahi AK, Acharya SK, Jameel S, Panda SK. Etiological role of hepatitis E virus in sporadic fulminant hepatitis. *J. Med. Virol.* 1994; **42**: 133–7.

95 Arora NK, Nanda SK, Gulati S *et al.* Acute viral hepatitis types E, A, and B singly and in combination in acute liver failure in children in north India. *J. Med. Virol.* 1996; **48**: 215–21.

96 Hamid SS, Jafri SM, Khan H, Shah H, Abbas Z, Fields H. Fulminant hepatic failure in pregnant women: Acute fatty liver or acute viral hepatitis? *J. Hepatol.* 1996; **25**: 20–7.

97 Sanyal MC. Infectious hepatitis in Delhi (1955–56): A critical study. Observations in armed forces personnel. *Indian J. Med. Res.* 1957; **45** (Suppl.): 91–9.

98 Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. *Am. J. Med.* 1981; **70**: 252–5.

99 Ticehurst J, Popkin TJ, Bryan JP *et al.* Association of hepatitis E virus with an outbreak of hepatitis in Pakistan: Serologic responses and pattern of virus excretion. *J. Med. Virol.* 1992; **36**: 84–92.

100 Krawczynski K, McCaustland K, Mast E *et al.* Elements of pathogenesis of HEV infection in man and experimentally infected primates. In: Buisson Y, Coursaget P, Kane M, eds. *Enterically-Transmitted Hepatitis Viruses.* Tours: La Simarre, 1996; 317–28.

101 Clayson ET, Myint KS, Snitbhan R *et al.* Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J. Infect. Dis.* 1995; **172**: 927–33.

102 Aggarwal R, Naik SR. Faecal excretion of hepatitis E virus. *Lancet* 1992; **340**: 787.

103 Nanda SK, Ansari IH, Acharya SK, Jameel S, Panda SK. Protracted viremia during acute sporadic hepatitis E virus infection. *Gastroenterology* 1995; **108**: 225–30.

104 Arankalle VA, Chadha MS, Chobe LP, Nair R, Banerjee K. Cross-challenge studies in rhesus monkeys employing different Indian isolates of hepatitis E virus. *J. Med. Virol.* 1995; **46**: 358–63.

105 Uchida T, Suzuki K, Iida F *et al.* Animal model, virology and gene cloning of hepatitis E. *Gastroenterol. Jpn.* 1991; **26** (Suppl. 3): 148–51.

106 Ticehurst J, Rhodes Jr LL, Krawczynski K *et al.* Infection of owl monkeys (*Aotus trivirgatus*) and cynomolgus monkeys (*Macaca fascicularis*) with hepatitis E virus from Mexico. *J. Infect. Dis.* 1992; **165**: 835–45.

107 Bradley DW, Krawczynski K, Cook EH *et al.* Enterically transmitted non-A, non-B hepatitis: Serial passage of disease in cynomolgus macaques and tamarins and recovery of disease-associated 27–34 nm viruslike particles. *Proc. Natl Acad. Sci. USA* 1987; **84**: 6277–81.

108 Longer CF, Denny SL, Caudill JD *et al.* Experimental hepatitis E: Pathogenesis in cynomolgus macaques (*Macaca fascicularis*). *J. Infect. Dis.* 1993; **168**: 602–9.

109 Tsarev SA, Emerson SU, Tsareva TS *et al.* Variation in course of hepatitis E in experimentally infected cynomolgus monkeys. *J. Infect. Dis.* 1993; **167**: 1302–6.

110 Soe S, Uchida T, Suzuki K *et al.* Enterically transmitted non-A, non-B hepatitis in cynomolgus monkeys: Morphology and probable mechanism of hepatocellular necrosis. *Liver* 1989; **9**: 135–45.

111 Corwin AL, Khiem HB, Clayson ET *et al.* A waterborne outbreak of hepatitis E virus transmission in southwestern Vietnam. *Am. J. Trop. Med. Hyg.* 1996; **54**: 559–62.

112 Khuroo MS, Dar MY. Hepatitis E: Evidence for person-to-person transmission and inability of low dose immune serum globulin from an Indian source to prevent it. *Indian J. Gastroenterol.* 1992; **11**: 113–16.

113 Joshi YK, Babu S, Sarin S, Tandon BN, Gandhi BM, Chaturvedi VC. Immunoprophylaxis of epidemic non-A, non-B hepatitis. *Indian J. Med. Res.* 1985; **81**: 18–19.

114 Arankalle VA, Chadha MS, Dama BM, Tsarev SA, Purcell RH, Banerjee K. Role of immune serum globulins in pregnant women during an epidemic of hepatitis E. *J. Viral Hepat.* 1998; **5**: 199–214.

115 Chauhan A, Dilawari JB, Sharma R, Mukesh M, Saroa SR. Role of long-persisting human hepatitis E virus antibodies in protection. *Vaccine* 1998; **16**: 755–6.

116 Tsarev SA, Tsareva TS, Emerson SU *et al.* Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc. Natl Acad. Sci. USA* 1994; **91**: 10 198–202.

117 Yarbough PO, Krawczynski K, Tam AW *et al.* Prevention of hepatitis E using r62K subunit vaccine: Full protection against heterologous HEV challange in cynomolgus macaques. In: Rizzetto M, Purcell RH, Gerin JL, Verme G, eds. *Viral Hepatitis and Liver Disease*. Turin: Edizioni Minerva Medica, 1997; 650–5.

118 Tsarev SA, Tsareva TS, Emerson SU *et al.* Recombinant vaccine against hepatitis E: Dose response and protection against heterologous challenge. *Vaccine* 1997; **15**: 1834–8.

119 Purdy MA, McCaustland KA, Krawczynski K, Spelbring J, Reyes GR, Bradley DW. Preliminary evidence that a trpE-HEV fusion protein protects cynomolgus macaques against challenge with wild-type hepatitis E virus (HEV). *J. Med. Virol.* 1993; **41**: 90–4.

120 He J, Hoffman SL, Hayes CG. DNA inoculation with a plasmid vector carrying the hepatitis E virus structural protein gene induces immune response in mice. *Vaccine* 1997; **15**: 357–62.