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Review

Hepatitis E virus: the current scenario

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SUMMARY

Hepatitis E infection, caused by the hepatitis E virus (HEV), is a common cause of acute hepatitis in developing countries with poor sanitation and hygiene. The virus is classified into four genotypes (1–4) with one serotype. Genotypes 1 and 2 exclusively infect humans, whereas genotypes 3 and 4 also infect other animals, particularly pigs. In endemic areas, large outbreaks of acute hepatitis caused by viruses of genotype 1 or 2 frequently occur due to fecal–oral transmission, usually through contamination of drinking water. With a high attack rate in young adults (aged 15–45 years), the disease is particularly severe among pregnant women (20–30% mortality). HEV appears to be a zoonotic disease, with transmission from pigs, wild boars, and deer, or foodborne. Chronic infections are rare, except in immunosuppressed persons, such as organ transplant recipients. A subunit vaccine has been shown to be effective in preventing the clinical disease, but is not yet commercially available. Our understanding of HEV has undergone major changes in recent years and in this article we review the currently available information with regard to the molecular biology, pathobiology, and epidemiology of HEV infection. We also review the current therapeutic interventions and strategies being used to control HEV infection, with emphasis on possible approaches that could be used to develop an effective vaccine against HEV.

1. Introduction

Viral hepatitis, caused by any of the five hepatotropic viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV), represents a major health problem worldwide. Among the viruses, HEV is now established as the major etiological agent of enterically transmitted non-A, non-B hepatitis. The first well-documented epidemic of hepatitis E infection in India was the epidemic of 1955–56 in New Delhi, affecting a total of 29 000 people; this occurred due to fecal contamination of drinking water. Although originally considered to be an epidemic of hepatitis A, retrospective testing of the stored sera from the affected patients suggested that a novel infectious agent was responsible.[1] Since the early 1990s, following the identification and sequencing of its etiological agent, the disease became known as hepatitis E and its agent as hepatitis E virus.[2] The letter 'E' stands for 'enteric', 'epidemic', or 'endemic', all of which are features that adequately describe the epidemiology of HEV. A high case fatality rate averaging around 20% in pregnant women, particularly in the third trimester, is a characteristic feature of HEV infection.[3,4] HEV is regarded as the major etiological agent of enterically transmitted non-A hepatitis in India.[5] HEV is responsible for both sporadic and epidemic outbreaks of acute hepatitis in developing countries, leading to a self-limiting disease. The global burden of HEV infection is more due to sporadically transmitted hepatitis E cases than to cases due to epidemic hepatitis E. Based on the 2010 global burden of diseases study, it has been estimated that as many as 20.1 million people were infected with HEV genotypes 1 and 2 in 2005, in nine regions. This represents 71% of the world's population, with 3.4 million symptomatic cases, 70 000 deaths, and 3000 stillbirths.[6] The death rate was higher among symptomatic pregnant women that among symptomatic non-pregnant women.[6]

HEV has been classified as the type species of the new genus *Hepevirus* in the family *Hepeviridae*.[7] Study of the molecular biology of HEV was significantly advanced with the establishment of an efficient cell culture system[8] and an effective vaccine,[9] but we still lack a reliable diagnostic procedure. However, anti-HEV antibody assays are widely available in European and Asian countries. Recent findings in HEV molecular biology, diagnosis, and pathogenesis are reviewed here.

2. Molecular biology

The genome of HEV is comprised of a single-stranded positive-sense RNA strand of approximately 7.2 kb and contains three open

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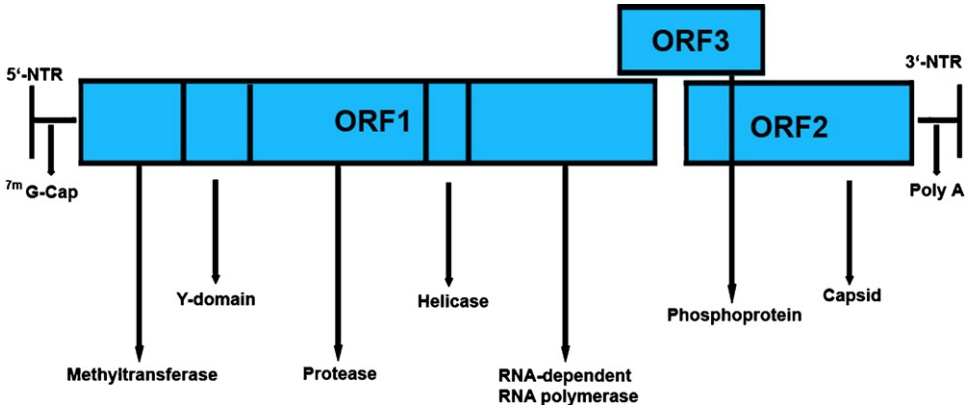

Figure 1. Genomic organization of the hepatitis E virus. The diagram depicts the organization of the genome (~7.5 kb). HEV is an RNA virus with three open reading frames (ORF 1–3) encoding nonstructural and structural proteins (NTR, non-translatable region).

reading frames (ORFs) that encode structural and non-structural proteins (Figure 1). ORF 1, present at the 5′ end, encodes a 1693 amino acid non-structural polyprotein. This polyprotein undergoes post-translational modifications to yield five different functional protein products, including a methyltransferase, a y-domain, a papain like cysteine protease, a helicase, and the RNA-dependent RNA polymerase. ORF 2, present at the 3′ end, encodes the 660–599 amino acid major capsid. ORF3 overlaps the other two ORFs and encodes a phosphoprotein that may help in replication and cytoskeleton synthesis. ORF1 contains a hypervariable region (HVR) that ranges from 557 to 641 amino acids. The HVR overlaps with the proline-rich sequence that is located between the N-terminus of the X domain and the C-terminal portion of putative papain-like protease domain. The HVR varies both in length and in sequence among different HEV strains.

Research at the Meng Laboratory has recently demonstrated that HEV can tolerate small deletions in the HVR and maintain its infectivity, suggesting that the amino acid residues in this region are dispensable for virus infectivity.[10,11] The genome sequence of HEV is relatively stable, although the genome of strains isolated from geographically distinct locations are generally more diverse. Based on whole genome sequencing, HEV has been characterized into four major genotypes: genotype 1 (Burmese-like Asian strains[12,13]), genotype 2 (a single Mexican strain[14]), genotype 3 (strains from rare endemic cases in industrialized countries and swine HEV strains worldwide[15,16]), and genotype 4 (variant strains from endemic cases in Asia and swine HEV strains in Asia[17,18]). All swine HEV strains identified thus far belong to genotype 1, 3, or 4.[19–21] The geographic distributions of the various genotypes is given in Figure 2. The recent discovery of novel lineages of HEV in rabbits,[22,23] rats,[24] and a wild boar[25] has further expanded the mammalian host diversity of HEV. These findings suggest that HEV has the capacity to be a zoonotic agent.

3. Pathobiology of the virus

Hepatitis E virus infection manifests both as epidemic and sporadic hepatitis in endemic disease areas. Since the first outbreak of HEV in 1955–56 in Delhi, many more outbreaks have been reported. During these outbreaks, attack rates of 1–15% (with a disproportionate number of cases seen in 15- to 40-year-olds) have been seen. The case fatality rate during epidemics was found to be between 0.2% and 4%, but an unexplainably high rate of fulminant liver failure was seen in pregnant women (mortality rate of around 10–20%). In India, 30–60% of all the sporadic cases of hepatitis are due to HEV infection.

The primary mode of transmission of HEV is fecal–oral. Evidence of fecal contamination of drinking water supplies has

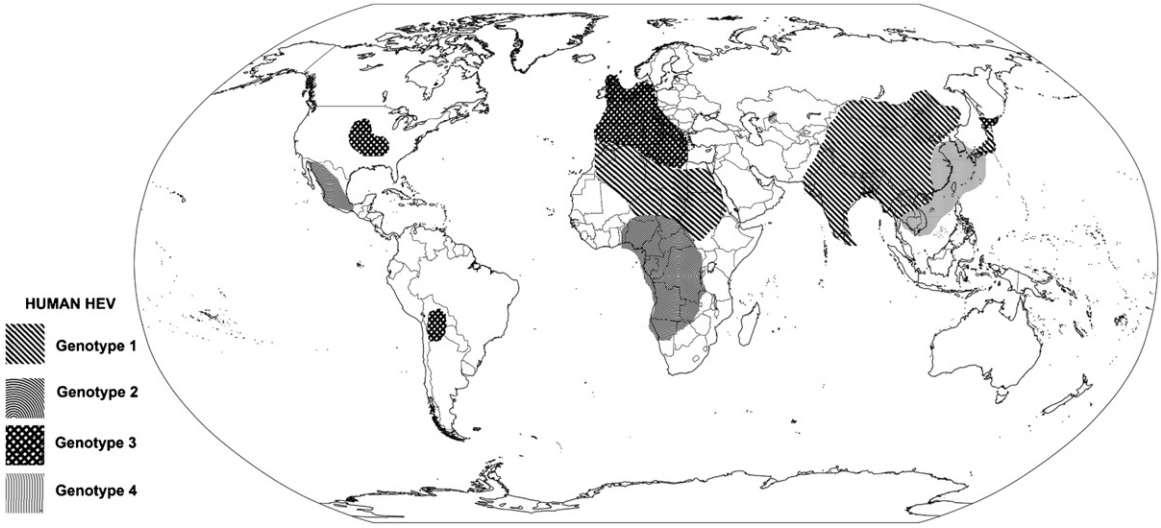

Figure 2. Geographical distribution of human hepatitis E virus genotypes. Map showing the geographical distribution of HEV genotypes among humans. Human HEV isolates are divided into four major genotypes (1–4), and each has its own geographical localization.

been associated with several outbreaks in India. Outbreaks occur most frequently during the rainy season, due to overflowing drains and the use of contaminated water for drinking.[26] Outbreaks have most often been associated with poor personnel hygiene, inadequate sanitation,[27] and an unsafe drinking water supply.[28] Overcrowding and refugee camps also exacerbate the problems.[29]

The clinical outcomes associated with HEV infection are quite diverse. HEV infection most commonly manifests as self-limiting, acute icteric hepatitis, which is indistinguishable from acute hepatitis caused by other hepatotropic viruses. In most cases, contact with HEV leads to an asymptomatic infection followed by spontaneous clearance of the virus, and only a minority of patients develop the symptomatic, icteric course of the disease.[6,9] In a proportion of patients, the illness is particularly severe and presents as fulminant hepatitis (acute liver failure). In animal studies, the viral inoculum dose determines the severity of the liver injury, and lower doses are associated with subclinical infection; whether this holds true for humans has not yet been studied.[30] Kumar et al. carried out a study aimed at characterizing the HEV isolates from acute viral hepatitis (AVH) and fulminant hepatic failure (FHF) patients based on the RNA-dependent RNA polymerase (RdRp) region.[31] They reported that all the isolates from acute cases belonged to subtype Ia and that the isolates from fulminant cases belonged to subtype Ic under genotype 1. This finding has raised the possibility of a correlation between disease severity and HEV isolate subtype. Liver abnormalities and coagulopathy rates that correlate with the damage to hepatocytes are seen in HEV-infected individuals. Resolution of biochemical abnormalities generally occurs within 1 to 6 weeks after the onset of illness, although some patients have a prolonged illness with prominent cholestatic manifestations. Chronic infection with HEV is virtually absent among healthy individuals, and has never been reported from HEV genotype 1 endemic countries. However, chronic hepatitis E after infection with HEV genotype 3 has been reported among persons receiving immunosuppressive treatments following organ transplantation.[32]

Research on neurological sequelae due to HEV infection is scarce, and reports come mainly from the Indian subcontinent, where genotype 1 infection is predominant. The role played by other genotypes in industrialized countries is unknown. Recently, Kamar et al. detected HEV RNA in the cerebrospinal fluid (CSF) of four patients out of seven studied for neurological complications due to HEV infection.[33] Although we need more such studies to confirm these findings, clinicians should strongly consider the possibility of HEV infection in patients with neurological disorders and especially in those patients who also have liver abnormalities as indicated by blood tests. In a separate study, the same group highlighted a possible relationship between neurological symptoms and HEV infection in a kidney transplant patient.[34] They were also able to show the existence of HEV quasispecies in the serum and CSF isolated from the patient.[34]

HEV infection can become chronic hepatitis in immunocompromised patients. Several reports have recently been published on the involvement of HEV in chronic infection, especially in solid organ transplant cases.[35] In endemic areas, HEV infection occurring in patients with pre-existing chronic liver disease of any etiology may lead to superimposed acute liver injury and clinical presentation of acute chronic liver disease. Such patients may be at higher risk of a poor prognosis. Kamar et al. evaluated the use of pegylated interferon alpha-2a (peg-IFN-α2a) in the treatment of chronic HEV in solid organ transplant (liver and kidney) cases and observed a sustained virological response after 3 months of treatment.[36,37] Although peg-IFN-α2a can be used to effectively treat chronic HEV infection after liver transplantation, peg-IFN-α2a use for kidney transplant patients is contraindicated. For this reason, Kamar et al. assessed the antiviral effect of ribavirin monotherapy in patients with chronic HEV infection following a kidney transplant. They observed that ribavirin monotherapy inhibited the replication of HEV in vivo and led to normalization of the levels of alanine and aspartate aminotransferase; however further studies are required to determine the optimal duration of ribavirin therapy.[38] A combination therapy of peg-IFN-α2a and ribavirin has been reported to be helpful in the clearance of HEV. Clearance was associated with an improvement in patient symptoms, a normalization of liver function tests, and a reduction in inflammation and fibrosis of the liver in a patient with chronic HEV/HIV-1 co-infection.[39] Gerolami et al. evaluated the use of ribavirin to treat severe acute HEV infection in non-immunocompromised patients and observed a rapid improvement in liver function tests concurrently with a decrease in HEV RNA levels in serum.[40]

4. Epidemiological patterns, prevalence, and mode of transmission

The incubation period of HEV in human volunteers after oral exposure is normally 4–5 weeks. A more variable incubation period of 2–10 weeks has been reported during hepatitis E outbreaks in which the time of water contamination was known. Research among non-human primates has shown a direct association between infective dose and disease severity, but an inverse relationship to the incubation period.[41] Four major routes of transmission of HEV infection have been reported. These are: (1) fecal–oral transmission due to contamination of drinking water supplies; (2) foodborne transmission; (3) transfusion of infected blood products; and (4) vertical transmission. Of these, transmission through contaminated drinking water seems to be the most common route. However, in cases in non-endemic regions and sporadic cases in disease-endemic regions, it is often not possible to establish the route of acquisition of infection. Distinct patterns of epidemiology have been seen in the geographical regions where hepatitis E is endemic compared to where it is non-endemic. In areas of endemic disease, epidemics of hepatitis E are more frequent and are usually separated by a few years. Such outbreaks have been observed in China, the Indian subcontinent, southeast and central Asia, the Middle East, and the northern and western parts of Africa. These outbreaks are usually large, and several hundred to several thousand persons are affected. Overall attack rates during hepatitis E outbreaks range from 1% to 15%, with males outnumbering females in most of them. Unlike several other enterically transmitted infections, person-to-person transmission of HEV is uncommon.[42] In hepatitis E endemic regions, the presence of HEV viremia among healthy blood donors and subsequent transmission of this infection to transfusion recipients has been documented. An anti-HEV IgG prevalence rate of 7.8% to 45% has been reported in volunteer blood donors in endemic countries, in contrast to 1–4% in industrialized countries.[43] Baylis et al. reported the presence of HEV in plasma fractionation pools in Sweden, Germany, and the USA.[44] All these findings raise the possibility of HEV transmission via the transfusion of contaminated blood products. Although the spread of HEV infection through contaminated food is possible, few outbreaks related to foodborne transmission have been reported from disease-endemic areas. This may be due to a relatively long incubation period, which makes it difficult to establish a relationship between consumption of a particular food and the occurrence of disease. The role of zoonotic transmission of HEV in endemic regions remains unclear. Hepatitis E has been reported in homosexual men,[45,46] which suggests a sexual mode of transmission. Data from endemic areas are scarce, however Bali et al. reported the spread of hepatitis E in an active group of male homosexuals in a village in north India.[47]

5. Possible zoonotic agent

While humans are generally considered to be the natural host for HEV, there is evidence to suggest that HEV also acts as a zoonotic virus. In a study carried out in China, the overall prevalence of anti-HEV IgG in the general human population was found to range from 32% among individuals who had frequent contact with swine to 21% among individuals whose contact was rare. The overall prevalence of anti-HEV in swine older than 3 months is 82%.[48] These data suggest that swine infections contribute to the high prevalence among people who have frequent direct exposure to animals. In the USA, a significant association of HEV seropositivity and animal contact has been found in humans who have pets in the home.[49] These data do not necessarily indicate that HEV infections in these people are exclusively zoonotic and may rather reflect a greater exposure of pet owners to environmental contamination from different sources, including human waste.

Recent research by different groups also suggests that foodborne HEV transmission is possible in relation to the ingestion of raw or undercooked meat and offal from swine.[50,51] If so, it would be expected that a source is infected with a single strain of virus and that this strain is transmitted to a recipient—therefore, viral genomic sequences identified in the two hosts should be identical or nearly identical. Although few foodborne transmissions have been documented, they provide genetic proof of HEV strains shared between an animal source and a human host. For example, the genetic analysis of the HEV strains in the livers of infected pigs sold at one local grocery store in Hokkaido, Japan, showed that some HEV variants found in porcine organs were identical to the HEV genotype 3 and 4 strains recovered from the local human cases.[52] More direct evidence for foodborne transmission was obtained by Tai et al., who reported a hepatitis outbreak among family members and some of their friends after they had eaten uncooked deer meat. Epidemiological studies and comparison of the RNA sequences of the HEV samples obtained from these patients and from a frozen portion of the suspect deer meat showed that they were all infected with HEV genotype 3.[53] Another study examined the HEV RNA sequences from wild boars, a deer, and four patients who had contracted hepatitis E after eating raw deer meat. The sequences displayed a sequence similarity of 99.7%. Although direct transmission from deer to humans could not be established, the sequence similarity suggested interspecies HEV transmission between boar and deer, as well as possible foodborne transmission to humans.[54] In another report, a woman whose husband was a boar hunter had eaten boar meat from one of his kills and became ill with hepatitis E. HEV RNA sequences from her serum and the consumed boar meat displayed a sequence similarity of 99.95%, confirming transmission of HEV from the boar to the woman.[55] Additional evidence of foodborne transmission was obtained from Hokkaido, Japan, where HEV genotype 4 strains were recovered from a series of sporadic hepatitis E cases from 2004 to 2009 where the isolates exhibited a high degree of sequence similarity.[56,57] With all these findings, it is now possible to say that HEV (especially genotypes 3 and 4) can and often does act as a zoonotic virus infection.

6. Diagnosis

Diagnosis of hepatitis E infection relies on laboratory abnormalities in liver enzyme and liver function tests, such as elevated serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, and gamma-glutamyltransferase. Abnormalities in liver function typically coincide with the onset of clinical symptoms. All these factors indicate a severe hepatic synthetic defect and extensive hepatocellular necrosis. Histopathological changes in the liver during acute infection include focal necrosis and modest inflammation. Cholestatic hepatitis is common and a 'pseudoglandular' alteration of hepatocyte plates has been noted. Since cases of hepatitis E are difficult to distinguish clinically from other types of acute viral hepatitis, the diagnosis is made by blood tests that detect elevated HEV antibody levels. HEV infection elicits both IgM and IgG. The IgM anti-HEV response is rapid, occurring about a month after infection and peaking at the onset of biochemical abnormalities and/or symptoms. There are a number of commercial enzyme immunoassays available for the detection of IgM and IgG anti-HEV in serum, although there is considerable variability in their sensitivities and specificities. This lack of consistency makes comparison of the diagnosis of HEV infection using different tests difficult. Drobeniuc et al. conducted a pan-genotypic validation of six commonly available IgM assays and found that only two of these assays had sensitivity and specificity above 95%.[58] HEV RNA can be detected in both blood and stool at the peak of the acute serological response. Reverse transcriptase polymerase chain reaction (RT-PCR) can be used to detect HEV RNA in serum and stool, but unfortunately such tests are not widely available in commercial laboratories. If laboratory tests are not available, then epidemiological evidence may help in establishing a diagnosis. The World Health Organization has established a number of international standards (ISs) for nucleic acid amplification (NAT)-based assays for several bloodborne viruses for use in blood and plasma safety checks.[59] Baylis et al. carried out the first study comparing the performances of these assays for the detection of HEV RNA and investigated the suitability of different HEV strains for the development of a candidate WHO IS.[60] Four virus strains – genotypes 3a, 3b, 3f, and 4c – were used for this study, and it was determined that any of these could be used as a potential strain to develop into an IS. As genotype 3 viruses have the widest distribution worldwide, they are a good candidate for WHO IS. More such studies involving other HEV strains are needed to establish a WHO IS for NAT-based assays for all HEV genotypes.

7. Treatment and control strategy

As fecal–oral transmission is the predominant mode of transmission of HEV infection, measures aimed at proper treatment and safe disposal of human excreta, the provision of a safe drinking water supply, and improvements in personal hygiene form the keystones of its prevention. Previous attempts at preventing hepatitis E by administering normal immune globulin manufactured from plasma obtained in the areas where HEV is endemic were unsuccessful,[61] or results were uncertain.[62] As the populations in regions where HEV is endemic have a relatively low prevalence and titer of anti-HEV, it is perhaps not surprising that unscreened batches of normal immune globulin may not contain protective quantities of anti-HEV antibodies.

Several recombinant proteins corresponding to the HEV capsid protein have been shown to induce specific antibodies in animals and to protect against liver injury following subsequent challenge with the virus.[63] In addition, an HEV DNA vaccine has also been shown to induce serum anti-HEV production in cynomolgus macaques and to protect against re-challenge with a heterologous HEV strain. As a potential human HEV vaccine, a 56 kDa truncated HEV ORF2 protein has been produced from a recombinant baculovirus that forms virus-like particles. This vaccine has undergone safety and efficacy studies in humans. In the phase I trial, this recombinant protein was given in an alum-adjuvant formulation and induced the production of anti-HEV among healthy volunteers in a dose-dependent manner.[63] In the subsequent phase II–III efficacy trials, nearly 2000 volunteers from the Nepalese Army who lacked detectable anti-HEV antibodies

received either 20 mg of alum-adjuvant recombinant HEV protein or a matched placebo. Each treatment was given as three doses (at 0, 1, and 6 months) and volunteers were followed up for more than 2 years.[64] Clinically overt acute hepatitis E occurred less frequently among vaccine recipients who completed the three-dose schedule than among placebo recipients, with a vaccine efficacy rate of 95%. A lower efficacy rate of 86% was observed after administration of two doses of the vaccine. Further studies are needed on the safety of this vaccine for pregnant women, children, and certain other groups, such as persons with chronic liver disease. The study focused on clinical disease rates and did not look at the HEV infection rate; thus it remains unclear whether the use of the vaccine will reduce rates of transmission of HEV in a community. Also, it was found that, by the end of the follow-up duration, the titers of anti-HEV had declined significantly and that nearly 44% of subjects had antibody titers below the level considered protective. Thus, further studies are needed to determine the duration of protection afforded by this vaccine. Unfortunately, this vaccine has not yet reached the market. In disease-endemic regions where the vaccine is likely to be most useful, high production costs are likely to prevent its widespread adoption. Recently, a Chinese group prepared another hepatitis E vaccine, named the HEV 239 vaccine. It contains a truncated HEV capsid protein (corresponding to amino acids 376–606) expressed in *Escherichia coli* that has been purified and adsorbed on aluminum hydroxide suspended in buffered saline.[65] In a human study, all the volunteers who lacked anti-HEV seroconverted in 1 month after receiving three doses (20 mg each at 0, 1, and 6 months, respectively).[66] After the second dose, new HEV infections were less frequent in the vaccine recipients than in the control subjects, indicating that the vaccine had a protective effect. A phase III trial is currently in progress. The exact role of the HEV vaccines remains unclear. In non-endemic regions, a vaccine would be useful for residents who are planning travel to an HEV endemic area, while in endemic areas a vaccine would be useful for pregnant women and persons with pre-existing chronic liver disease where there is a likelihood of severe disease following HEV infection. Many factors, such as cost considerations, the duration of protection afforded by the vaccines, and their ability to interrupt the transmission of infection, will decide whether HEV vaccines should be used for the general population in endemic regions.

8. Future perspectives

The last few decades have seen major advancements in the development of control strategies for HEV. Further efforts are needed to develop newer and more effective antiviral agents using advanced techniques like phage display or small interfering RNA (siRNA) -based interference systems; this should be possible in the near future. With the use of the phage display technique, novel proteins or peptides specific for HEV can be detected and characterized. These could then be used either as a therapeutic vaccine or to develop a diagnostic test. With current advancements in the field of reverse genetic systems, we are now able to generate infectious clones for many RNA viruses—this also seems possible for HEV. The development of infectious clones would provide us with working constructs, which would be very useful in obtaining valuable information about the pathogenesis of HEV.

The central epidemiological question in hepatitis E research is its unexplained high severity in pregnancy, for which there are still no concrete answers. Pregnant monkeys experimentally infected with HEV do not show this effect, and studies in human patients have not adequately addressed this issue. Recent reports have increased our understanding of the role of ORF3 in HEV pathogenesis. The importance of this protein in HEV pathogenesis is further reinforced by the lack of experimental infection in monkeys by an ORF3-null virus. The role of this protein in HEV pathogenesis needs to be investigated.

9. Conclusions

HEV is the leading cause of non-A, non-B enterically-transmitted acute viral hepatitis in India. As the risk factors associated with the transmission of sporadic hepatitis E are currently unknown, we cannot recommend prevention strategies at this time. Specific attention should be given to persons at higher risk of severe illness by giving priority to the prevention of infection in this vulnerable group during HEV outbreaks. With the current global burden of epidemic and sporadic hepatitis E, the high mortality among pregnant women, the severity of autochthonous hepatitis E, and the threat caused by the widespread prevalence of HEV infection in different populations, expansion of epidemiological studies, especially clinical trials of promising hepatitis E vaccine candidates, should be pursued in endemic areas.

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