

Hepatitis E vaccines – Current scenario

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Introduction

Hepatitis E is an important global public health disease with worldwide annual estimate of 20 million new infections, of which around 3 million cases are symptomatic and 56,600 people die of hepatitis E-related disease [1]. It is also known as epidemic associated non-A, non-B (ENANB) hepatitis or enterically transmitted NANB hepatitis as it is transmitted by faeco-oral route and it is distinct from the hepatitis A (HAV) and hepatitis B virus (HBV). The existence of Hepatitis E virus (HEV) came to notice during the retrospective analysis of the large scale outbreak in Delhi in the year 1955-56 affecting around 29,300 people [2]. Similar studies for the existence of non-A non-B hepatitis virus were reported from Kashmir valley and different parts of India [3,4]. In 1983, Balayan and co-worker's demonstrated the viral particle by immune electron microscopy in faecal sample of the research team workers who themselves ingested the pooled faecal extract of the patients with non A non B hepatitis [5]. Since then many such outbreaks from different geographical regions have been reported, mainly from developing countries where general sanitation and hygiene conditions are poor and safe drinking water is not available adequately. However, even in developed countries, HEV has been known to cause outbreak and sporadic cases due to zoonotic transmission especially from swine [6].

HEV is a non-enveloped, spherical virus particle with 32-34 nm size with icosahedral symmetry. Due to its morphological similarity with Norwalk virus it was placed under Calciviruses [5]. However with the use of cryo-electron microscopy and crystallography, the structure of HEV was well defined. The International committee for Taxonomy of viruses (ICTV) has now placed the virus under the family *Hepeviridae* and genus *Hepevirus* [7]. HEV has a positive sense single stranded RNA of 7.2 kb in length. The viral genome consists of a short 5' terminal capped end with three open reading frames (ORF 1-3) and a 3' end with adenosine stretch (Figure 1) [8]. The ORF 1 codes for the viral non-structural proteins which have a role in capping of viral genome and viral replication. The ORF 2 encodes for the capsid protein, which is an important antigenic component of the virus and ORF 3 codes for the proteins that have an important role in the genetic assembly and virion release [9]. The virus is resistant to acid, bile acids and heating at 56°C for 1 hr.

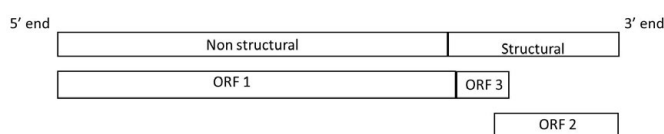


Figure 1. Genomic organization of hepatitis E virus (HEV) showing the open reading frames (ORF). Adapted from Pérez-Gracia MT *et al.* [8].

HEV has four genotypes and single serotype. Each genotype differs from each other by 25% or more and has its own geographical distribution and host susceptibilities [10,11]. Genotype 1 and 2 are known to infect human and non-human primates and are found in developing countries like Asia, Africa and Latin America and are predominately transmitted by faeco oral route [10]. On the other hand, genotype 3 and 4 are predominantly acquired by zoonotic route especially from the intake of contaminated food and have been recovered from swine, deer, rabbits, rats, cattle and others apart from humans [6,10,12].

The HEV infection is acquired by oral route from contaminated water or food [13]. However there are reports of transmission by body fluids of infected animals, infected blood products and mother to child transmission [14,15]. Following the entry of virus in the gut, the virus reaches the liver via portal vein. Of the several receptors recognized, heparan sulphate proteoglycans serves as the low affinity receptor promoting the virus contact with the hepatocytes [16]. HEV replicates in the protoplasm of the hepatocytes and later is released in the blood and bile, leading to viraemia and faecal shedding of virus [17].

Incubation period of HEV infection ranges from 2 weeks to 2 months. The clinical symptoms and signs of HEV cannot be differentiated from the other cases of hepatitis [10,18]. The usual clinical features include fever, malaise, vomiting, jaundice, headache and abdominal pain. The individual affected are usually young adults in genotype 1 and 2 infections, whereas the older age groups and immunosuppressed patients are affected in case of infection caused by genotype 3 and 4. The males are more affected than females [7,18]. The disease is known to progress more severely in patients infected with both human and swine strain of HEV especially in patients of poor countries [19]. The disease usually presents in any of the three forms. The acute viral hepatitis with a self-limiting course, acute liver failure especially in case of pregnant women and patients with existing liver disease. Recently, chronic HEV infection has also been documented transplant recipients and in some patients on chemotherapy and Human immunodeficiency virus (HIV) co-infection [19]. Chronic hepatitis is usually associated in case of infection caused by HEV genotype 3. The pregnant women are known to develop acute liver failure due to high viral load and hormonal abnormalities in pregnancy [20]. The severity of infection in pregnancy increases with each passing

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trimester. The risk of foetal loss is high in first trimester of pregnancy. The overall mortality in HEV infection is around 1% in immunocompetent patients, 20% in pregnancy and around 30% in patients with chronic liver disease [21,22]. HEV can also present with subclinical infections with low viral load in case of outbreak settings and endemic areas. These subclinical infections serve as the reservoir of HEV in the local community [23].

The diagnosis of HEV infection can be made in early phase of infection by HEV RNA detection in stool and serum samples [24]. The viraemia and faecal shedding usually starts 3 weeks after infection and 1 week prior to the disease onset. The viraemia diminishes with time even though the faecal shedding may persist for days to weeks [11]. HEV RNA detection targeting the conserved region between HEV genotype is useful especially in the early phase, in patients with immunosuppression who may not mount an antibody response. Viral load determination may be important in cases of chronic infections caused by HEV to determine the response to treatment [25]. Recently, HEV antigen detection has also been found to be an early diagnostic marker especially in the first 3 days of infection when patient may not have detectable anti-HEV antibodies [26]. This has a distinct advantage over HEV RNA detection that it can be done in a routine serology laboratory. The routine diagnosis of current HEV infection rests on the detection of IgM anti-HEV antibodies by ELISA which usually becomes detectable by the end of first week of disease onset and may last up to 5 months [27,28]. The IgG anti-HEV antibodies peak 2-4 weeks after onset of disease, and is known to persist for years in low levels [11]. In case of outbreak settings, the use of dried blood spots (DBS) samples for detection anti-HEV IgM antibodies has also been shown to be useful [29]. Most of the acute HEV infections resolve on their own and may need occasional symptomatic treatment. Antiviral therapy like ribavirin and interferon are used in cases of chronic infections. In recent studies, sofosbuvir has been found to be effective in inhibiting HEV replication and can be used as an adjunctive to ribavirin therapy for chronic hepatitis E infections [30].

Hepatitis E vaccine

Though, both HAV and HEV share common characteristic of enteric transmission by faeco-oral route, but both are quite different in their biological properties. There is successful vaccine available against HAV, but no such vaccine is still available against HEV. However, there is a hope to develop successful vaccine against HEV as only single serotype is present and passive immunization with high titre anti-HEV plasma or serum has been shown to provide protection against different genotypes in non-human primates when challenged with HEV [31].

Need for vaccine

HEV infection is a disease of global concern with one-third of the world's population being infected. The prevalence reported is just the tip of an iceberg, where the actual load of the disease is under reported. HEV is capable of causing outbreaks and epidemics. There is possibility of reinfection in the communities where HEV is known to be endemic and in patients with altered immune response [18].

Target groups

The HEV vaccine may be beneficial in pregnant females and in patients with pre-existing chronic liver disease who are prone to develop acute liver failure. Also, there may be some role of the vaccine in transplant recipients who are predisposed to develop chronic liver disease.

Vaccine designing

Among the several HEV viral proteins, the ORF 1 and ORF 3 encoded proteins are less accessible to antibodies and don't completely neutralize the virus [32,33]. The ORF2 encoded viral capsid proteins have been shown to be highly conserved, have better neutralizing ability, generate long lived antibodies and have shown high cross reactivity with different HEV genotypes and hence are better candidates for vaccine development [34].

Due to the difficulty in growing HEV in cell lines, the antigen targets are prepared by recombinant technology [35]. Two systems, *Escherichia coli* and insect cells from *baculovirus* vectors have been effective to express the HEV viral proteins. The protein forms expressed are usually the truncated proteins as the full-length proteins are relatively hydrophobic and insoluble. Till date two vaccines have been studied extensively, there are *Escherichia coli* derived vaccines and *Baculovirus* expressed vaccines.

Escherichia coli derived vaccine

The *E. coli* derived viral proteins of 23kDa (aa 394-606) and 30 kDa (aa 368-606) prepared by the Wantai Biological pharmaceutical Co in China have been studied in trials [36]. Initially, the preclinical trial studies were done in rhesus monkeys which were given two vaccine doses at an interval of 4 weeks. The infection challenge was given in two sets (high-dose and low-dose), and samples were taken 3 weeks after the 2nd dose. The protective efficacy was observed to be 75% and 100% in high dose and low dose challenged monkeys respectively. The vaccine also showed cross protection to genotype 4 in addition to genotype 1 [36].

In China, HEV 239 *E. coli* vaccine was given to 612 seronegative subjects. The seroconversion was reported to be 100% when 3 doses of 10 µg were given and 98% when 2 doses of 20 µg were given. With good safety and efficacy, this vaccine entered into Phase 3 trial [37]. The Phase 3 trial was carried out in China under the sponsorship of Ziamen Innovax Biotech. A total of 112604 healthy male and female individuals were divided into vaccine and placebo groups [38]. Three doses of 30 µg were given intramuscularly to adults (16-65 years) and active HEV infection was followed up till 13 month after vaccination. The efficacy was found to be 100% (CI 9.1%-100%) after the second dose and 100% (CI, 72.1%-100%) after the third dose. The vaccine had few mild side effects [38]. This vaccine with the name "HEV 239" or Hecolin[®] Innovax is licensed in China for use in patients of age group 16-65 yrs who are prone to HEV infection like pregnant women, traveller to endemic countries, military recruits and animal food handlers [38]. The vaccine efficacy has been found to be good in patients aged 16-65 years for genotype 1 and 4; however its efficacy in genotype 2 and 3 and in extreme age groups needs to be clearly evaluated [39].

Baculovirus derived vaccine

The Baculovirus expressed genotype 1 viral proteins of 62 kDa; 56 kDa and 53 kDa were initially evaluated in in-vitro and in-vivo experiments. The 56 kDa (aa112-278) protein showed better results and was prepared by GlaxoSmithKline (GSK), Belgium for trial studies [40]. In the preclinical trial, two different formulations with different vaccination dose schedules were evaluated in rhesus monkeys [41]. Following the vaccination, the monkeys were subjected to challenge by one of the three genotype viruses (genotype 1, 2 and 3 strains). The protective efficacies were noted to be 100% and 75% in two dose and single dose regimen groups respectively. The vaccine provided

protection against all genotypes. The placebo group developed hepatitis in 75% of the cases [41].

The phase I clinical trial of *Baculovirus* vaccine was carried out by Walter Reed Army Institute of Research in US army volunteers. The individuals with age of 18-50 years were administered 3 doses of four (1, 5, 20, 40 µg) formulations. The seroconversion rates were 89% to 95% in higher dose formulations [42]. The phase II trial was carried out in Nepal army volunteers where HEV infection is endemic [43]. A total of 1794 healthy young adults, mostly male individuals were evaluated for the efficacy in vaccine and placebo groups. A total of 3 doses each of 20 µg strength were given at 0, 1, 6 months intramuscularly and followed up for clinical infection till 2 years after vaccination. The seroconversion rate at 1 month after 3rd dose was 100%. The vaccine efficacy was found to be 95.5% (CI, 85.6%- 98.6%) and 85.7% (CI, 16%-98.2%) in individuals who received 3 doses and 2 doses respectively. This vaccine has been found to be effective in regions where HEV is endemic [43].

The need of the hour is that these vaccines should be introduced in to the market and made available at reasonably low price to the community [44]. Till date, both the vaccines have been found to be effective in different setups and have been extensively reviewed by the Global Advisory Committee on Vaccine Safety (GACVS) [39]. The HEV 239 vaccine was considered to be better vaccine based on the phase III trials, however the data on safety in patients with pre-existing liver disease, in patients <16 year and >65 years and cross protection for genotype 2 and 3 is lacking. Due to insufficient information about safety and efficacy of this vaccine, WHO does not recommend it for the routine use in the national immunization programmes. However, it recommends the use HEV 239 vaccine in outbreak settings and pregnant women, where the risk of complications and mortality is higher. However the use of HEV 239 vaccine in case of travellers and outbreak associated health care and relief workers needs to be decided on the basis of cost – benefit ratio [39]. WHO in view of the global burden of HEV, has come up with initiatives to fill in the gap in information, so that in the years to come a proper vaccination programme is available.

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