Review

Type E Hepatitis: State of the Art

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ABSTRACT

Hepatitis E (HE) occurs predominantly in tropical and semitropical countries in the form of sporadic cases or epidemics of variable magnitude. In industrialized countries, only imported sporadic cases of HE have been reported, with little evidence of human-to-human transmission. HE resembles hepatitis A clinically and epidemiologically but affects young adults rather than children, showing a higher mortality rate in pregnant women. HE virus (HEV) shares many characteristics of the caliciviruses, although it is genomically distinct from this family of viruses.

New diagnostic tests have been developed, based on the use of recombinant or synthetic antigens that are analogues of HEV structural proteins. These have been applied to determine the prevalence of antibodies (anti-HEV) in various epidemic and nonepidemic settings. The prevalence of anti-HEV antibodies was unexpectedly low even in endemic areas. A low but constant rate of seropositivity was observed among normal individuals permanently living in nonendemic countries of Europe and North America, while an elevated rate of anti-HEV was found in certain groups of patients and risk groups. This situation as well as other unresolved problems, such as the possible involvement of nonhuman reservoirs, the existence of subclinical forms, and potential prevention strategies, need further investigation.

Key Words: *diagnostics, epidemiology, hepatitis E, laboratory models, serologic surveys, viral hepatitis*

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Type E viral hepatitis, formerly known as enterically transmitted non-A, non-B hepatitis, is an acute, icteric, self-limited disease widely spread in many tropical and semitropical countries. In industrialized countries with moderate climates, only occasional imported cases of this disease are reported.

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Hepatitis E (HE) is sometimes described as a new disease.[1] The disease may have been recently described or identified, but it is not a newly emerging infectious disease. It is likely that HE was a disease of antiquity subsumed within the common term "infectious jaundice."

HISTORICAL BACKGROUND

As far back as the Middle Ages, when hygienic conditions for the vast majority of humanity were poor, many enteric viral and nonviral infections were constantly present in communities. By analogy with modern epidemic situations, it could be expected that human populations were virtually 100% immune to hepatitis A virus (HAV) from early childhood. Hence widespread epidemics of jaundice disease that are noted in historical records most probably were associated with hepatitis E virus (HEV) rather than with HAV, particularly if high attack rates among adults, a distinctive feature of HE, are taken into account.

In the 11th through the 13th centuries, several outbreaks of severe jaundice occurred among the crusaders during their marches to the Middle East. The descriptions of the clinical manifestations of these epidemics resemble those seen with HE.[2] At the end of the eighteenth century, a disease similar to HE affected many soldiers and officers of Napoleon's army involved in military campaigns in Egypt and Syria.[3] It was also suggested that HE may have caused the epidemics of so-called "catarrhal jaundice," which were common in Europe and elsewhere before the turn of the last century, but became restricted to less developed countries later (a pattern that is being repeated by hepatitis A now).[4] In the early 1920s in western Ukraine, fatalities in pregnant women, associated with acute hepatitis, another distinctive feature of HE, were documented among cases of jaundice; in this period, laboratory methods could only detect bacterial or parasitic pathogens, so it was not possible to identify HEV as the etiologic agent in these outbreaks.[5]

As soon as reliable diagnostic tests for serologic confirmation of hepatitis A and acute hepatitis B had been developed, it became evident that large water-borne outbreaks and a number of sporadic cases in India and other tropical countries were not etiologically associated with either HAV or hepatitis B virus (HBV). Several outbreaks were thoroughly studied,[6–8] and the existence of an

enterically transmitted non-A, non-B hepatitis distinct from hepatitis A (i.e., representing a separate nosologic entity) was strongly suggested. Later this disease was designated as hepatitis E (for epidemic, endemic, enterically transmitted).[4]

Virologic studies culminated in identification of a viral agent responsible for HE infection and disease; this relied on visualization of virus-like particles (VLPs) in the excreta of typical HE patients, including an experimentally infected human volunteer, and transmission of the disease to nonhuman primates.[9] For several years, immune electron microscopy (IEM) remained the only diagnostic tool for confirmation of etiologic involvement of HEV. Although the IEM was a rather tedious and time-consuming procedure, it permitted more accurate estimation of the number of HE outbreaks in endemic areas.[10-13]

In 1990, the HEV genome was successfully cloned in a prokaryotic system.[14] Shortly after, a variety of HEV-specific antigens in the form of recombinant proteins and synthetic peptides were produced. These antigens could be used in conventional enzyme immunoassays (EIAs) as an immobilized reagent, making the test itself suitable for efficient large-scale serologic surveys.[15]

THE VIRUS

The etiologic agent of HE is a small, spherical, nonenveloped virus. In IEM, the HEV particles (virions) look like round objects 27 to 34 nm in diameter often coated with a "halo" of attached antibody molecules. When the concentration of antibody is not too high, cup-shaped indentations on the virion surface and spikes on its contour can easily be seen, providing an argument for similarity between the HEV and the viruses found in the Caliciviridae family. Other characteristics of HEV also compatible with those of caliciviruses are shown in Table 1.[16,17,17a] It was repeatedly noted that HEV virions are not stable and lose their integrity after routine laboratory procedures, such as freeze-thawing, sedimentation in salt solutions, and dialysis.[18] This feature is rather unusual for an agent which, under natural conditions, survives in the environment despite exposure to many unfavorable conditions, such as elevated temperature, solar radiation, and osmotic extremes.

The HEV exists as a single serotype; that is, the HEV isolates from different geographic regions share the same conservative antigenic pattern. In usual serologic tests, the HEV particles and HEV-specific recombinant antigens react equally well with sera from convalescent patients irrespective of geographic origin of either viruses or sera. Furthermore, monkeys infected with one HEV isolate acquire resistance to superinfection with the HEVs from distinct outbreaks.[19]

Based on molecular cloning experiments, the HEV genome is a single-stranded positive-sense RNA of approximately 7.5 kb polyadenylated at 3′ terminus.[20] The genome is flanked with short noncoding sequences at 5′and 3′ termini and encompasses three partially overlapping open reading frames (ORFs). The larger one (ORF1), of approximately 5.0 kb, is located at the 5′ end, and it encodes for nonstructural elements with enzymatic activities. The second largest (ORF2), of 2.0 kb, at 3′ end controls the synthesis of major structural protein(s). The smallest ORF3, with 0.3 kb, occupies the intermediate position and expresses a protein of unknown function (Figure 1). It is assumed, although not yet proven, that the messages for nonstructural and structural proteins of HEV are generated separately through a hypothetic mechanism of transcription regulation.[21] Comparison of nucleotide sequences in a genome fragment encoding for putative RNA-directed RNA polymerase (RDRP) revealed 87 to 94% homology between the Asian strains of HEV, and as low as 77% homology between the strains from different continents (e.g., Burmese versus Mexican

Table 1. Comparative Characteristics of Hepatitis E Virus and Some Caliciviruses

	HEV	*Caliciviruses*[16,17]
Virion morphology		
Size (diameter) (nm)	27–34	32–38
Surface indentations	Dim	Sharp
Flotation properties		
S_{20w}	183	170–185
Buoyant density, g/cm^3		
CsCl	1.35	1.33–1.39
KTar/gly	1.29	
Stability		
In lab conditions	Labile	Labile
In the evenironment	Stable	Stable
Virion composition		
RNA	Single-stranded positive sense ~7.5 kb	Single-stranded positive sense ~8.2 kb
Maor protein	55–70 kDa	60–71 kDa
Antigenic pattern	Single serotype	Several distinct serotypes (specific to host species) with considerable cross-reactivity
Host range	Humans, non-human primates; other vertebrates	Interspecies transmission is not infrequent; potential for zoonosis

Adapted from Balayan, 1994.[17a]

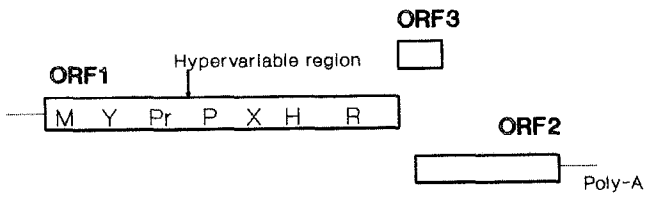

Figure 1. Schematic representation of hepatitis E virus genome. ORF1: Nonstructural proteins: M = methyltransferase; Y = domain Y; Pr = papain-like cysteine protease; P = proline "hinge;" X = domain X; H = helicase; R = RNA replicase. ORF2: Structural protein(s). ORF3: A protein of unknown function. — = Noncoding sequences.

isolates).[22] Such genetic divergence does not go beyond the strain variations normally observed within the members of a single virus genus.

The HEV shares many common morphologic and physicochemical characteristics of caliciviruses. However, the nucleotide sequences of the HEV genome fragment corresponding to RDRP was not homologous to any entries in the GenBank database,[14] including those of the prototype calicivirus strains, and no similarities were found between the HEV and caliciviruses in genome composition, although their overall genomic organization seems to be the same.[21] A distant degree of sequence homology was seen with rubella virus belonging to the *Rubivirus* genus within the togavirus family and scarcely studied plant furoviruses. Although the final taxonomic definition of the HEV remains to be established, it can be tentatively assumed that this virus is a single member of a novel virus genus that may be further placed in either calici- or togavirus families or perhaps will represent a distinct virus family.

LABORATORY MODELS

Over ten species of nonhuman primates appeared to be susceptible to experimental HEV infection, showing some features similar to those of the human disease. Wild-caught cynomolgus macaques were predominantly used in the experiments, though rhesus monkeys, African vervet monkeys, marmosets, tamarins, and chimpanzees were also effectively inoculated. It was shown recently that Old World monkeys, trapped in their natural habitat, occasionally have antibodies to HEV (anti-HEV).[23]

There are five criteria according to which the HEV infection in these animals can be recognized:

1. Pathologic elevation in the level of liver enzymes, alanine aminotransferase (ALT) and isocitrate dehydrogenase (ICD), temporally related to the infection,
2. Specific morphologic alterations (necroinflammation) in hepatic cells,
3. Detection of HEV antigen(s) in the liver by a fluorescent antibody technique (FAT),
4. Fecal shedding of HEV particles, and
5. Anti-HEV immune response, particularly persistence of class M antibody.

In addition, a test for HEV RNA in blood (i.e., the viremic status, using the polymerase chain reaction [PCR]) was applied to monitor the HEV infection more accurately.[24] No jaundice has been observed in HEV-infected monkeys or apes.

The biphasic profile of liver enzyme elevation is characteristic of experimental HEV infection in nonhuman primates (Figure 2). Usually a "minor" peak appears as early as 6 to 12 days after inoculation, followed by a week-long period of normalization of enzyme levels and then by a new, often more pronounced, "major" peak. In the interval between these two peaks, the HEV particles are regularly visualized in the stool and the viral antigen can be detected in liver cells with specific fluorescent antibody. It is hard to say whether this enzyme profile takes place in human HE infection too, since the first peak obviously corresponds to the preclinical stage when persons are unlikely to seek medical attention.[25,26]

Experimentation in nonhuman primates provided a valuable source of HEV in the form of relatively pure suspensions of viral particles in the bile derived directly from the gall bladder of infected monkeys (e.g., this material was used for molecular cloning of HEV).[14] Pedigreed serum panels composed of pre-inoculation, acute, and convalescent sera were also obtained from infected monkeys and further employed for the validation of serologic assays and immunoscreening of cDNA libraries.[22]

The HEV does not grow well in conventional cell culture systems. Attempts to isolate the virus from patients' excreta by inoculation and passages in a variety of cell cultures has been unsuccessful. However, under certain experimental conditions, HEV appears to be capable of propagating in cultivated cells. In our previous studies, this was achieved by co-cultivation of primary liver

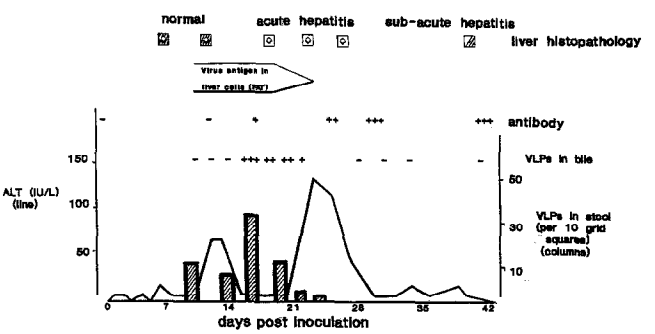

Figure 2. Typical time-course of HEV infection in monkeys (cynomolgus macaques). ALT = alanine aminotransferase; FAT = fluorescent antibody technique; VLPs = virus-like particles.

and kidney cells derived from HEV-infected monkeys at the peak of disease, with fresh cells of continuous lines (FRhK-4 and 4647). The persistent infection was soon established and further maintained in serial passages; the presence of HEV in these systems could be determined by cDNA hybridization, fluorescent antibody technique (FAT), and inoculation of persistently infected cells into susceptible monkeys.[27] Later the HEV RNA in persistently infected cells also was detected by a PCR technique (unpublished results). The Chinese isolates of HEV were shown to grow in selective cell lines producing the marked cytopathogenic effect; no additional adaptation of HEV was required for the efficient propagation of the virus in these cells.[28,29]

Evidence is accumulating that, in addition to primates, nonprimate vertebrates also may be susceptible to HEV infection. In 1990, it was shown that HE can be reproduced in young (sub-adult) domestic pigs after intravenous inoculation with human HEV isolates.[30] The time-course and other parameters of experimental infection in these animals were similar to those observed in monkeys; some infected pigs even developed jaundice, manifested as icteric skin and sclera at weeks 3 to 4 after inoculation. This finding was confirmed by the detection of HEV RNA sequences and anti-HEV antibody in stool and serum specimens, respectively, collected from free-roaming swine in the Kathmandu Valley of Nepal.[31] Among other domestic animal species, lambs were found to be susceptible to HEV infection, at least under experimental conditions.[32]

Hepatitis E virus also has been transmitted to laboratory rats.[33,34] Although no clinical disease was observed in these animals, evidence for virus replication in liver and nonliver (spleen, lymph nodes, small intestine) cells as well as pathologic alterations (signs of necroinflammation) in liver tissue was obtained. From the epidemiologic point of view, it is noteworthy that HEV infection can occur in wild rodents as well. In one study, anti-HEV antibody was detected in some wild-trapped rodents living in close proximity to potable water sources in a rural area endemic for HE.[33] All these findings imply possible involvement of domestic and perhaps certain wild animals in the perpetuation of HEV. This has not been thoroughly investigated, and whether HE is a zoonosis remains to be determined.

CLINICAL COURSE

Clinical aspects of HEV infection have been extensively reviewed.[4,18,35] Hepatitis E resembles hepatitis A as well as other forms of acute viral hepatitis, although some hepatologists claim that they can differentiate HE on the basis of minor symptoms and clinical biochemical findings.

The liver seems to be the only target organ for HEV infection, hence extrahepatic manifestations of the disease are rare. The incubation period ranges from 15 to 40 days, with a median of 35 days being more protracted than that observed with hepatitis A. The onset of symptoms is usually abrupt; practically all the signs and symptoms of the disease appear simultaneously. Normalization occurs within 2 to 3 weeks, except for severe fulminant cases. The disease is usually self-limited with no chronic sequelae; however, the elevated levels of liver enzymes (e.g., ALT) sometimes can persist for an additional 1 to 2 weeks.

Histopathologic studies of liver tissue obtained by a needle biopsy provide no pathognomonic features for HE, simply yielding results consistent with acute viral hepatitis, often with abundant cholestasis.

The main clinical symptoms of HE in the order of frequency of occurrence are shown in Figure 3. When the disease progresses toward the severe fulminant forms, which occurs at a rate of 1-3% in adults and up to 20 to 30% in pregnant women, the symptoms of increasing intoxication due to liver dysfunction begin to dominate the clinical picture. In pregnant women this status frequently leads to abortion or premature delivery, which in turn aggravates the disease.

Asymptomatic or subclinical forms of HE are not well defined. Recently, it became possible to identify HEV infection by detecting anti-HEVs of IgG and IgM class capable of reacting with recombinant or synthetic HEV antigens. In suspected HE cases, the rate of anti-HEV seropositivity varied significantly, depending on the composition of HEV-specific antigens, the geographic regions where the cases occurred, and the class of antibody that was detected.[36]

GEOGRAPHIC SPREAD AND EPIDEMIOLOGY

In the past, when HE was recognized only by the appearance of distinct water-borne epidemics or constant

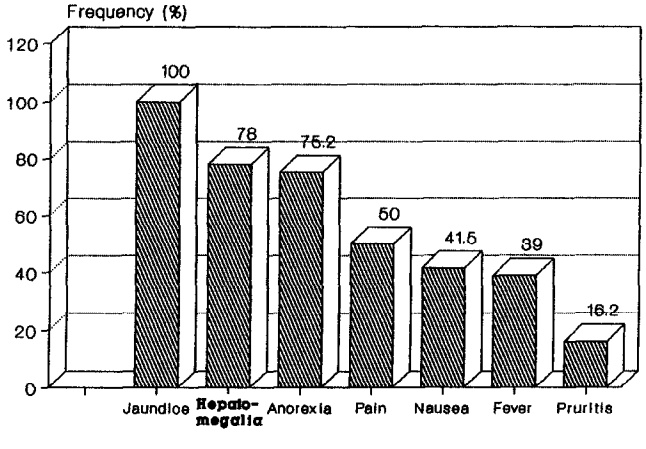

Figure 3. Clinical symptoms in adult patients with hepatitis E. Modified from Purcell and Ticehurst,[4] with permission.

Table 2. Main Epidemiologic Features of Hepatitis E Virus Infection

1. Fecal-oral mode of transmission
2. Frequent occurrence of epidemics and outbreaks associated with consumption of sewage-polluted water
3. Geographic distribution: tropical and subtropical countries throughout the world
4. Seasonal pattern: increased incidence during or after rainy seasons, in Southeast Asia; during late autumn, in Central Asia (Confederation of Independent States and China)
5. Age-specific prevalence: highest attack rate in young and middle-aged adults (15–40 y)
6. Higher incidence of infection in adult males versus females; no sex preponderance in children
7. Limited spread of infection around source case in households, communities, or hospitals (low secondary attack rate)

presence of typical sporadic cases, the area of HE distribution included many developing countries located in the tropical belt. In addition, large foci of the disease were found to exist in the Central Asia republics (territory of the former USSR) as well as in northern and central provinces of China. Among the countries that reported the occurrence of HE were India, Pakistan, Nepal, Myanmar (formerly Burma), Indonesia, China, Ethiopia, Sudan, Somalia, Algeria, Senegal, Ivory Coast, and Mexico. These countries share poor socioeconomic and hygienic conditions that facilitate the spread of HEV through contaminated water and, perhaps, household contacts. It should be noted, however, that the above picture was obtained on the basis of available information from the public health sources and laboratory investigation of clinical specimens; hence, a significant portion of HE morbidity obviously remained under-reported.

The application of serologic tests with recombinant or synthetic HEV-specific antigens has revealed the presence of anti-HEV in human populations practically in every country in which a proper survey has been performed, including the industrialized countries where clinically overt HE occurs only as imported sporadic cases. It is further discussed below whether these findings are compatible with the real distribution of HEV infection.

The main epidemiologic features of HE are summarized in Table 2. These features focus on endemic areas, without taking into account the rare but constant presence of anti-HEV seropositivity in the rest of the world. Table 3 compares the characteristics that usually are used to describe the endemic versus nonendemic areas.

SEROLOGIC SURVEYS

In 1992, an enzyme immunoassay (EIA) based on the use of recombinant antigens, analogues of the HEV structural proteins, was introduced.[15] A variety of EIA variants were recommended thereafter; they differed with regard to the nature and composition of HEV-specific antigens ultimately aimed at the maximal detection of anti-HEV in patients and healthy subjects. At present, there are three groups of antigens included in the EIA: (1) recombinant proteins expressed in *Escherichia coli*,[15] (2) recombinant proteins expressed in insect cells,[37] and (3) combinations of synthetic peptides linked to a large-molecule carrier.[38] None of the tests developed to date contain native whole-virion HEV antigens.

The antigen most commonly used consists of a 327 amino acid fragment from the 3′ terminus of ORF2, presumably a component of an HEV structural protein, and a sequence of 123 amino acids representing the full length of ORF3; both are derived from the Burmese strain of HEV and are expressed in *E. coli*. It is included in a commercially available test kit as a solid-phase reagent. Anti-HEV antibody, detected with this reagent, was found to be present in a significant portion of suspected HE patients.[39,40]

Irrespective of the test employed, anti-HEV was detected in normal healthy individuals who were permanent residents of areas endemic for HE; however, the rate of anti-HEV seropositivity was low and never reached the figures regularly observed for the antibody to HAV, an etiologic agent of another enterically transmitted viral hepatitis.[40-42] For example, in the hyperendemic area of Tadjikistan, 8.5% of healthy subjects were seropositive for anti-HEV.[38] The rates for anti-HEV seropositivity were 4 to 5% in India,[43] 2.8% in Thailand,[43] and 20.2% in China.[44] In our previous studies of sera collected from healthy subjects and nonhepatitis patients in another

Table 3. Hepatitis E: Characteristics of Endemic Versus Nonendemic Areas

	Endemic	*Nonendemic*
Incidence of HE	High	Low (or none)
Epidemiologic manifestations	Outbreaks of variable magnitude	Only imported sporadic cases
General sanitation	Poor	Adequate
Spread through contaminated water sources	Frequent	Absent
Personal hygienic habits	Only in certain social groups	In great majority of population
Nonhuman HEV reservoirs	Very probable	If exist, do not play essential role
HE reporting system	Often under-reported	Registered all suspected cases
Climatic conditions	Hot climate	Moderate climate

hyperendemic area (Kirghizstan), anti-HEV seroprevalence was 4.6%, whereas anti-HAV seroprevalence was 93.0%.[45]

The paucity of anti-HEV seropositives in normal human populations of endemic countries remains unexplained. One could speculate that (1) anti-HEV antibody does not persist for a long period and falls below detectable levels soon after the acute infection, (2) the HEV causes predominantly a clinically overt disease and sub-clinical or "silent" forms rarely occur—this would be atypical for an enterically transmitted infection, or (3) the serologic tests miss a significant portion of the spectrum of anti-HEV antibodies.

Unexpectedly, a low rate of anti-HEV was also found in the nonendemic industrialized countries located mainly in the moderate climate belts; as a general tendency this rate increases in the north to south direction. In the Netherlands, 1.1% of blood donors appear to be anti-HEV sero-positive,[46] in the United Kingdom, 1.0%,[47] in France, 0.9%[47] (in French pregnant women, 2.6%[48]), in Spain, 2.2%,[47] in Israel, 2.6%,[49] in Switzerland, 4.5%,[50] to mention only a few of these examples. In a nonendemic area of Russia (Moscow) only one (0.6%) of 165 observees was anti-HEV seropositive whereas 43.7% were seropositive to HAV.[45] Among blood donors in the United States, 2.1% had anti-HEV antibodies,[15] a prevalence that is ten times higher than that for hepatits C virus.[51]

An increased rate of anti-HEV seropositivity was demonstrated in certain groups of patients with liver pathologies that, according to present knowledge, are not directly associated with the HEV infection, such as chronic autoimmune liver disease,[52] chronic hepatitis, and primary liver cancer.[53] Anti-HEV seropositives were found among hemophiliacs,[54] donors rejected from blood donations due to elevated levels of ALT,[50,53] and HIV-infected homosexual men,[55] more often than in the general population.

There are three hypothetical, though not mutually exclusive explanations for these phenomena: (1) the inadequacy of methods employed for the detection of anti-HEV, (2) a situation where the same virus causes a certain pathology in one climatic zone and a completely different disease in another (e.g., the Epstein-Barr herpesvirus is the cause of infectious mononucleosis in moderate climates and Burkitt's lymphoma in the sub-Saharan Africa), or (3) the existence of an unrecognized agent capable of cross-reacting with antibodies to HEV.

CONTROL AND PREVENTION

Improvement of hygienic standards and general sanitation in endemic areas could result in decreasing HE morbidity. In particular, measures to prevent contamination of water sources are expected to be effective; instruction in the proper treatment of potable water can be recommended as well. It is important that pregnant women should be admitted to intensive care units as soon as HEV infection is suspected.

There are some prospects for the development of a subvirion HEV vaccine from genetically engineered immunogens. Preliminary evidence has shown that the HEV-ORF2 product expressed in *E. coli* and fused to trpE protein can protect susceptible monkeys from challenge with wild-type HEV.[56] An alternative immunogen, consisting of full-length ORF2 protein expressed in insect cells, has been suggested recently as a suitable candidate vaccine for HE (Tsarev SA, personal communication).

Among the target groups that should be considered for vaccination are females of pre-child bearing age in endemic areas and persons from nonendemic areas planning to visit tropical countries.

CONCLUSIONS

Despite the rapid progress in understanding the etiology and pathology of HE, many areas require futher research. The unusual patterns of anti-HEV seroprevalence stimulate further studies to determine whether the paucity of anti-HEV in endemic areas is associated with a higher susceptibility to HEV infection in the majority of the population. The rare, but significant presence of anti-HEV in the industrialized countries signals the need for regular vigilance. Additionally, the possible role of domestic or wild animals in HEV transmission should be further elucidated before a general strategy for prevention of HE in endemic countries can be formulated.

Information concerning the emergence of a third enterically transmitted hepatitis agent is awaiting confirmation.[57,58] This may further complicate healthcare in many countries. However, the rapid growth in the understanding of HEV demonstrates the benefit of collaboration among scientists, clinicians, and public health experts.

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