Review

A Review of Hepatitis E Virus†

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ABSTRACT

Hepatitis E virus (HEV) is a major cause of outbreaks and sporadic cases of viral hepatitis in tropical and subtropical countries but is infrequent in industrialized countries. The virus is transmitted by the fecal-oral route with fecally contaminated drinking water being the usual vehicle. Hepatitis resulting from HEV infection is a moderately severe jaundice that is self-limiting in most patients. Young adults, 15 to 30 years of age, are the main targets of infection, and the overall death rate is 0.5 to 3.0%. However, the death rate during pregnancy approaches 15 to 25%. Death of the mother and fetus, abortion, premature delivery, or death of a live-born baby soon after birth are common complications of hepatitis E infection during pregnancy. Hepatitis E virus is found in both wild and domestic animals; thus, HEV is a zoonotic virus. The viruses isolated from swine in the United States or Taiwan are closely related to human HEV found in those areas. The close genetic relationship of the swine and human virus suggests that swine may be a reservoir of HEV. In areas where swine are raised, swine manure could be a source of HEV contamination of irrigation water or coastal waters with concomitant contamination of produce or shellfish. Increasing globalization of food markets by industrialized countries has the potential of introducing HEV into new areas of the world. The purpose of this review is to cover certain aspects of hepatitis E including the causative agent, the disease, diagnosis, viral detection, viral transmission, epidemiology, populations targeted by HEV, and the role of animals as potential vectors of the virus.

Hepatitis E virus (HEV) is the causative agent of more than 50% of sporadic cases of viral hepatitis in tropical and subtropical countries with inadequate sanitation and overburdened public health infrastructures *(5, 22, 178)*. In addition, the virus is a source of waterborne epidemics in these countries. The death rate due to hepatitis E infection in the general population of HEV endemic countries is 0.5 to 3%; however, the mortality rate in pregnant women can be as high as 15 to 25% *(109)*.

The earliest confirmed hepatitis E outbreaks occurred in India during the 1950s *(30)*. This led to the assumption that HEV was a relatively new disease agent. However, epidemics of enterically transmitted hepatitis with high fatality rates in pregnant women have been reported as far back as the 1850s *(28, 45, 136)*, suggesting that HEV has been a cause of hepatitis for a longer period than previously thought.

Hepatitis E in developed countries is generally restricted to persons who travel to an endemic area *(101)*. Increased travel for business and pleasure to HEV endemic areas and increased globalization of the world marketplace *(72, 101)* may lead to the introduction of hepatitis E infection to new geographical areas.

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THE CAUSATIVE ORGANISM

The virion of HEV is a nonenveloped sphere of 27 to 34 nm diameter, and its genome is a single-stranded, positive-sense RNA molecule approximately 7.5 kb in length. The RNA is polyadenylated at the 3′-terminus and is capped at the 5′-end with 7-methylguanosine *(85, 178)*. The taxonomic position of HEV is not clear and the virus is presently unclassified *(119)*.

Under laboratory conditions, HEV is quite labile. Disintegration of the virus occurs in the presence of the high salt concentrations utilized during purification by CsCl equilibrium gradient centrifugation, during concentration by ultracentrifugation, during storage at temperatures between −70 and +8°C, and during freezing-thawing *(22, 175, 179)*. Although HEV is labile under laboratory conditions, these conditions are not necessarily germane to the stability of the viruses under environmental conditions. Harrison *(65)* suggests that a virus spread by the fecal-oral route must survive environmental conditions and must survive passage through the acidic milieu of the host's stomach. In addition, the finding of HEV RNA in sewage *(83, 131)* indicates that the viruses can survive in hostile environments.

The complete genomic sequence has been determined for several strains of HEV. The genome is organized into three separate but overlapping open reading frames (ORFs). The largest, ORF1, is located at the 5′ end and codes for nonstructural proteins responsible for viral genome repli-

cation and protein processing *(97, 98, 154, 179)*. The second largest ORF (ORF2), at the 3′ end, codes for and regulates the synthesis of the major structural protein, the viral capsid. The smallest ORF in HEV is ORF3, and it overlaps with both ORF1 and ORF2. The protein expressed by ORF3 is a phosphoprotein with unknown function *(98, 154, 159, 164, 179, 181, 182)*. The proteins expressed by both ORF2 and ORF3 are highly immunogenic *(22)* and have been used in serological diagnosis of HEV.

Genotypic diversity of HEV. The distinctive genotypes of HEV correlate with their geographical origin. Phylogenetic analysis indicates that there are at least three distinct genotypes of the virus based on geographical origin: Asia-Africa, United States, and Mexico *(145, 163)*. Recent studies by Hsieh et al. *(74)*, Schlauder et al. *(145)*, and Wang et al. *(174)* suggest that other geographically restricted genotypes of HEV may be present.

A number of workers have suggested that HEV may be a zoonotic virus *(22, 65, 112)*. Meng et al. *(117, 118)* found HEV in pigs from the midwestern United States that showed nucleotide sequence similarity (92 to 98%) to human strains of HEV of the U.S. genotype. HEVs isolated from pigs and humans in Nepal were closely related in terms of RNA nucleotide sequence *(163)*. Similarly, Taiwan pig and human isolates of HEV were closely related with 97% identity at the nucleotide level *(73)*. These results suggest that for a particular geographical region, HEVs from pigs and humans are comparable. A worldwide survey to determine the nucleotide sequences of swine HEV, as well as for HEV nucleotide sequences in other animals, should be undertaken. A comparison of those sequences with human HEV sequences should provide the needed information about potential HEV reservoirs.

Serotypic diversity of HEV. The geographical distinctness of HEV is limited to the nucleotide sequence. Amino acid sequences determined for different genotypes of HEV are highly conserved and would indicate that there is only one serotype of the virus *(163)*. Immunoassays for the capsid protein encoded by ORF2 from a single strain of HEV are capable of identifying HEV antibody in individuals regardless of the infecting genotype *(179)*. For example, an enzyme-linked immunosorbent assay (ELISA) and a fluorescent antibody assay utilizing recombinant antigens from Burma and Mexico strains of human HEV *(53, 129, 137)* were used to detect HEV antibody and HEV viral antigen in a patient diagnosed with hepatitis in the United States *(99)*. Nucleotide sequence analyses indicated that the patient had a strain of HEV that was genetically distinct from the Asia-Africa and Mexico genotypes. This genetically distinct strain, designated as US-1, was placed in the unique U.S. genotype of HEV *(143)*. Even though the serological detection systems were based on antigens from strains from Asia-Africa or Mexico genotypes, these systems were capable of detecting the presence of the genetically distinct U.S. strain of HEV.

THE DISEASE

Hepatitis E in most patients is a moderately severe icteric (i.e., jaundice) disease with a self-limited course similar to that seen in hepatitis A virus (HAV) infections. Cholestatic (i.e., obstructive) jaundice is a feature of HEV infection. Cholestatic jaundice, due to obstruction of the bile ducts, leads to the elevation of serum bilirubin. The incubation period of hepatitis E for humans ranges from 15 to 60 days with a mean of 40 days. Icterus is usually accompanied by malaise, anorexia, abdominal pain, liver enlargement, vomiting, and fever. The jaundiced patient displays yellowish skin, scleral icterus (i.e., yellowing of the white of the eye), dark urine, and light tan-colored feces *(101, 109, 158)*. Chronic sequelae are not observed, because there is no evidence of liver chronicity and/or cirrhosis of the liver following infection. The case-fatality rate for hepatitis E infection ranges from 0.5 to 3% except in pregnant women where it ranges from 15 to 25% *(101, 109, 158)*. The infective dose of HEV for humans or animals is currently unknown due to the lack of an in vitro method for cultivating the virus.

Hepatitis E virus RNA has been detected in both the feces and sera of humans infected with the virus *(38, 42, 125)*. The virus has been detected in the bile of monkeys *(14, 126, 155)* and is probably present in the bile of humans. Viremia is seen during hepatitis E infections *(38, 42, 125, 144)*. The duration of viremia is normally about 2 weeks *(42)*; however, viremia lasting 39 to 112 days after appearance of icterus has been reported in a few symptomatic adult patients *(42, 125)*. Arora et al. *(20)* reported viremia of 105 to 110 days duration in infected children; viremia was also demonstrated in an asymptomatic child.

The hepatitis E infection rate is two to four times that of the clinical disease rate. Thus, subclinical or anicteric disease is more common than overt hepatitis E with jaundice *(43)*. Infected individuals without apparent illness, but shedding virus, may be reservoirs of virus between epidemics and may contribute to sporadic infection by contaminating water or food and by person-to-person spread. Chadha et al. *(35)* studied the persistence of anti-HEV IgG in 37 individuals who had hepatitis E. All individuals who had been infected during hepatitis E epidemics were positive for IgG anti-HEV at 5 years of follow-up. Two persons with sporadic hepatitis E were still seropositive at 8 and 12 years. None of these individuals showed signs of jaundice during the follow-up periods, which suggested that reinfection had not taken place. The results obtained by Chadha et al. *(35)* indicated that antibody to HEV is long-lasting and protective.

TISSUE CULTURE

There have been very few reports of the use of tissue culture in HEV studies. Kazachkov et al. *(89)* demonstrated the persistence of HEV RNA and HEV antigen in serially passaged FRhK-4 (i.e., fetal rhesus monkey kidney) cells after cocultivation with primary kidney cells derived from HEV-infected monkeys. Viral persistence was maintained for at least 12 consecutive passages in FRhK-4 cells, thereby indicating that the virus had replicated because it was not diluted out by serial passage *(89)*.

The propagation of HEV in a human embryo lung diploid cell line was reported by Huang et al. *(76)*. Cytopathic

effects such as cell rounding and eventual cell destruction were seen in cell culture monolayers that had been inoculated with a stool sample from a hepatitis E patient. Viral production was monitored by immunoelectron microscopy *(76)*; however, the viral particles were not tested for the presence of HEV RNA or HEV proteins. Using A549 human lung carcinoma cells infected with HEV from Chinese sporadic cases, Huang et al. *(75)* determined that the viruses were nonenveloped RNA particles and not resistant to acid (pH 3.0) or heat (56°C/30 min). Electron microscopy indicated that the virion was round, 25 to 36 nm in diameter, and with an irregular surface. Partial genome determination of the Chinese virus indicated that it may represent a new HEV genotype *(75)*.

Tam et al. *(155)* demonstrated the replication of HEV in vitro by infecting normal primary cynomolgus macaque hepatocytes suspended in hormonally defined serum-free medium with the Burma strain of the virus. Because HEV is a positive-stranded RNA virus, the presence of newly replicated virus was shown by detection of both the negative and positive strands of HEV RNA in the infected liver cells *(155)*. Detection of positive-stranded RNA, but not negative-stranded RNA, in the culture medium indicated viral shedding; however, the physical presence of virus was not demonstrated. Only about 10^5 viral genomes/ml were produced in culture, which was too low for detection by electron or immunoelectron microscopy *(155)*. Using PLF/PLC/5 human hepatocarcinoma cells infected with HEV, the detection of negative replicative strands of the virus indicated HEV replication *(56)*. Cytopathic effects were not observed.

Improved detection techniques, vaccine development, and increased knowledge about HEV have been hampered by the lack of an adequate in vitro cultivation technique. Further study on the optimization of in vitro cultivation procedures will lead to great advances in the understanding of HEV.

DIAGNOSIS OF HEV INFECTION

Detection of HEV antibodies. ELISAs based on recombinant antigens expressed by ORF2 of HEV RNA were more sensitive for detecting anti-HEV IgG or IgM than were tests based on antigens expressed by ORF3 or on a combination of ORF2 and ORF3 antigens *(62)*. The ORF2-encoded protein contains six antigenic domains. Domains 1 and 6, located at the N and C termini, respectively, contain strong IgG and IgM antigenic epitopes. Recombinant proteins and/or synthetic peptides derived from these domains should prove useful as diagnostic targets for detection of anti-HEV antibodies in patients presenting with acute hepatitis *(90)*.

A chimeric antigen consisting of hepatitis B virus core particles combined with an epitope from the ORF2 C-terminal region of HEV was used to develop an ELISA particularly sensitive for the determination of IgM anti-HEV *(160)*. The ELISA based on the chimeric antigen was as effective as a commercial ELISA *(53)* for detection of anti-HEV IgG but was 1.7-fold more sensitive for detecting anti-HEV IgM than the commercially available ELISA. Using an antigen containing 267 amino acids from the carboxyl-terminal portion of ORF2 that is highly conserved in divergent HEV strains, Anderson et al. *(8)* have developed an ELISA that allows the detection and quantitation of acute, as well as convalescent-specific, IgG from patients infected with HEV. Western immunoblots using recombinant proteins from ORF2 and ORF3 *(105, 129)* or recombinant proteins from ORF2 *(78, 79, 104)* are a useful technique for the detection of antibodies against HEV.

Detection of HEV RNA. HEV RNA in serum, feces, and bile of infected humans or nonhuman primates has been detected by the use of reverse transcription-polymerase chain reaction (RT-PCR) *(3, 39, 82, 164, 171)*. Viremia and/or fecal shedding of HEV was detected by RT-PCR in patients with acute hepatitis lacking detectible anti-HEV IgM or IgA *(42)*.

TRANSMISSION

Transmission by water. HEV is transmitted principally by the fecal-oral route. Epidemics of hepatitis E that occur in HEV endemic countries are generally due to fecally contaminated water *(5, 109)*. Endemic areas, where large common-source epidemics and/or the constant presence of sporadic cases of hepatitis E infection are found, extend through many tropical and subtropical countries, including areas in Central and Southeast Asia, Northern and Western Africa, and a small part of Mexico *(135)*. These areas are also known for their impoverished social and economic conditions as well as for inadequate hygiene and sanitation *(22, 108)*. Conditions conducive to hepatitis E epidemics can arise if raw sewage is carried to water reservoirs during floods, monsoons, or seasons of heavy rains. Intermittent flow of water through leaky pipes that pass through polluted soils or sewage holes and accidental interconnection of sewage lines with drinking water pipes also result in contamination of drinking water *(92, 147)*.

Communities in Indonesia and Vietnam affected by hepatitis E outbreaks have been located on major rivers. These rural communities are characterized by inadequate sanitary infrastructures. A high incidence of HEV seropositivity was correlated with use of river water for drinking, cooking, personal washing, and disposal of human excreta *(47)*. In areas where river water was boiled before use in cooking or drinking, there was a significant decrease in anti-HEV IgG prevalence *(47)*. Thus, in Southeast Asia, river water is a primary source of HEV transmission. War refugees or people in urban slums who are living under crowded conditions and who lack sanitary facilities and safe water supplies are at increased risk for hepatitis E or other fecal-oral transmitted diseases and epidemics can become a common problem *(22, 92, 108)*. Sewage treatment in developing countries may not be adequate to inactivate or remove HEV. Jothikumar et al. *(83)* surveyed the incidence of HEV in sewage influents and effluents from three sewage treatment plants in Madras City, India. All sewage influent samples contained HEV. After treatment of sewage in these plants, removal of HEV ranged from 67 to 89%. The presence of other enteroviruses, as well as HEV, in the

treated effluents from these plants indicated that sewage treatment was not totally effective in removing enteroviruses. Using such treated water for drinking purposes or for processing foods may account for sporadic cases of HEV in some instances.

Person-to-person transmission. Direct person-to-person transmission of hepatitis E virus during epidemics plays a minor role in the spread of hepatitis, with an attack rate of <2.5% within households containing primary cases *(4, 26, 67, 78, 86, 111, 140, 151)*. Person-to-person transmission of HEV probably plays a minor role during large waterborne epidemics because the gross contamination of drinking water leads to a near-universal exposure of the population to that water.

In an examination of sporadic hepatitis E infections, Khuroo and Dar *(91)* found evidence of person-to-person spread. Household contacts of an infected index patient comprised 29% of hepatitis E cases, suggesting that secondary spread is important in sporadic infections. Person-to-person transmission of hepatitis E in a hospital setting also has been reported. Hospital personnel who were exposed to blood, feces, and other fluids during the birth of a child to a hepatitis E-infected woman, later became ill with hepatitis, apparently due to secondary transmission *(142)*. It is likely that person-to-person spread of HEV does occur, especially under poor hygienic conditions.

Transmission by food. It is probable that foods can act as vehicles for transmission of HEV, but no cases or outbreaks have been unequivocally shown to be due to food consumption. Foods washed or processed with HEV-contaminated water, produce irrigated with HEV-contaminated water, or shellfish harvested from HEV-contaminated waters could lead to disease if these foods are eaten raw or inadequately cooked. Cacopardo et al. *(33)* attributed acute hepatitis E occurring in eastern Sicily to consumption of shellfish obtained from fecally contaminated waters. In addition, raw or cooked foods that have been handled by an HEV-infected person with poor personal hygiene could be a source of virus. Hartmann et al. *(66)* reported a case of a Turkish national living in Germany who showed signs of recent hepatitis E infection that may have been foodborne. The patient had not traveled to Turkey in the preceding 16 weeks. The incubation period for hepatitis E is considered to be 2 to 9 weeks, and therefore, the patient probably did not have travel-related HEV. The patient did have a Turkish visitor who had brought ethnic foods as a gift. Hartmann et al. *(66)* speculated that the source of the patient's infection was the Turkish foods or person-to-person transmission by the visitor who may have been subclinically infected. However, there was no report of a clinical examination of the visitor.

Ishikawa et al. *(81)* described a case of hepatitis E transmitted by ingestion of a Chinese herbal medicine. The patient was a Japanese national who had traveled to China where he had obtained the herbal medicine. Analysis by RT-PCR of the virus isolated from the patient indicated that the virus was closely related to a Chinese strain of HEV. It is possible that the herbal medicine was the source of the virus.

Other forms of transmission. Using a sample of eight babies, Khuroo et al. *(93)* presented evidence that suggested HEV transmission from mother to fetus. Hepatitis E transmission by blood donation has been postulated by Sylvan and Hampl *(153)* and Wang et al. *(173)*; however, the number of individuals tested was small. In an Indian population, Arankalle and Chobe *(15)* found asymptomatic blood donors whose sera were HEV RNA-positive, indicating virus was present in the blood of the donors; however, there was no evidence that recipients of the blood became ill with hepatitis E.

There are a number of reports indicating that the prevalence of antibodies against HEV was higher in hemodialysis patients than in blood donors. In general, these patients did not show clinical manifestation of hepatitis E *(2, 18, 34, 52, 64, 113)*. Other studies indicate that the prevalence of hepatitis E is not increased in hemodialysis patients *(49, 59, 134)*. Psichogiou et al. *(134)* found that an apparent increased prevalence of anti-HEV IgG in hemodialysis patients was eliminated when the cases were compared to appropriate age and gender controls in the general population.

In a small study, Montella et al. *(122)* found that homosexual men infected with the human immunodeficiency virus were more likely to be hepatitis E-positive than human immunodeficiency virus-negative homosexual men. In contrast, other workers indicate that human immunodeficiency virus-infected patients are not any more at risk for hepatitis E than the general population *(27, 133, 161)*. Psichogiou et al. *(133)* also found that individuals with various sexually transmitted diseases were not more susceptible to hepatitis E than other population groups. Due to the limited numbers of individuals studied, unequivocal evidence that HEV transmission occurs via blood, sexual contact, or mother-to-fetus simply does not exist.

EPIDEMIOLOGY

HEV is transmitted by fecally contaminated water or food *(63)* and, to some extent, by person-to-person spread *(91)*. HEV is endemic in many developing countries of Asia and Africa, as well as elsewhere, particularly in those countries where weak economies have led to inadequate sanitation, unsafe water supplies, and lack of public health infrastructures *(108)*. Outbreaks of hepatitis E have been reported in Asia, Africa, and Mexico *(30)*. Hepatitis E outbreaks are most often waterborne and some outbreaks, notably in Burma, India, and Nepal, have resulted in several thousand hepatitis cases *(100)*. Hepatitis E is responsible for >50% of acute viral hepatitis in many underdeveloped countries *(6)*.

In endemic regions, hepatitis E is responsible for a large proportion of sporadic (i.e., nonepidemic) acute viral hepatitis cases. It has been estimated that 50 to 60% of sporadic cases of hepatitis in India are due to hepatitis E infection *(5)*. The mode of transmission for sporadic hepatitis E is probably fecal-oral similar to epidemic hepatitis E. While fecally contaminated water is the vehicle for ep-

idemics of hepatitis E, the vehicle in transmission of sporadic hepatitis E is not clear, but most probably water and/or food and person-to-person contact are involved. Sporadic cases of hepatitis E also may be due to contact with infected animals (see "Animals and HEV" below).

Immigrants to the United States from HEV endemic areas and travelers returning to the United States from areas where HEV is endemic have been diagnosed with acute hepatitis E *(29, 179)*. For example, acute hepatitis E was detected in travelers returning to the United States who had visited India, Mexico, and Nepal *(11, 21, 54)*. Smalley et al. *(150)* described acute hepatitis E in an Indian immigrant who was diagnosed with hepatitis within a week of his arrival in the United States. Ooi et al. *(127)* found that 1.7% of 211 HEV-seronegative U.S. citizens who traveled to HEV-endemic areas demonstrated HEV seropositivity when they returned to the United States. These individuals did not show clinical symptoms of hepatitis E.

Similarly, acute hepatitis E has been seen in travelers from other developed countries after visiting endemic and/or epidemic areas *(146)*. For example, acute hepatitis E was detected in travelers returning to the United Kingdom after visiting Bangladesh, China, Ethiopia, India, Nepal, Pakistan, and Saudi Arabia *(148, 149)*; in Dutch travelers who had traveled to Bangladesh, the Middle East, and Somalia *(180)* and in Taiwanese travelers returning after visits to mainland China *(177)*. Travelers should take appropriate action such as drinking boiled water and eating thoroughly cooked foods to prevent infection in their host countries. Clinicians should do a thorough history on individuals presenting with hepatitis who have traveled outside of the United States and on immigrants who present with hepatitis.

Prevalence of anti-HEV IgG in blood donors indicates a significant difference between industrialized and nonindustrialized countries (Table 1). The percent prevalence in industrialized countries varied from 0.0 to 3.3 (mean = 1.2), but the prevalence was higher in nonindustrialized countries and varied from 7.2 to 24.5% (mean = 15.2). Except for India, epidemics of hepatitis E have not occurred in any of the nonindustrialized countries listed in Table 1, but the high prevalence of HEV seropositivity would indicate that the virus is endemic in those countries. The seropositive blood donors from industrialized countries do not remember having clinical manifestations of hepatitis *(110, 113)*. It is not clear why a small fraction of individuals from developed countries are seropositive for HEV. A possible explanation may be that these individuals contacted subclinical infection from an animal reservoir (see below).

Recently, three cases of locally acquired acute hepatitis E were diagnosed in the United States. The first case was a patient, with no history of foreign travel, who was ill after returning to Minnesota from a trip to San Jose, California *(99)*. The patient's serum, tested by RT-PCR, was positive for HEV RNA. Sequencing of the viral RNA indicated that the virus diverged in RNA sequence significantly from the Mexico and Asia-Africa strains. The virus strain, US-1, was placed in a new genotype of HEV—the U.S. genotype *(143)*. The second patient had traveled to Cancun, Mexico, several weeks before his illness. The virus isolated from the patient, designated US-2, was closely related to US-1 and was significantly divergent from the Mexico and Asia-African strains *(58)*. Virus strains US-1 and US-2 had 92% nucleotide identity to each other, but the two U.S. strains were only about 74% identical to the Mexico and Asia-African genotypes *(58)*. The third hepatitis E case involved a patient who resided in California and had not traveled outside of the state. The patient had symptoms for hepatitis and was positive for anti-HEV IgM and IgG. The source of the infection was not identified *(162)*. In the case report, there was no mention of virus characterization, and therefore, the HEV genotype sequence of the infecting virus is not known. At the present time, the role of the U.S. genotype in terms of HEV seropositivity in blood donors from the United States is unknown.

A few cases of locally acquired sporadic hepatitis E infections have been reported for other countries not endemic for HEV including: Australia *(71)*, France *(48)*, Greece *(156)*, Italy *(183)*, New Zealand *(37)*, and the United Kingdom *(114)*. The presence of locally acquired hep-

TABLE 1. *Prevalence of anti-HEV IgG in blood donors*

Country (reference)	Number positive/total number (% positive)
Industrialized countries	
Australia (*121*)	1/279 (0.4)
Australia (*8*)	9/500 (1.8)
Germany (*178*)	0/200 (0.0)
Germany (*95*)	5/250 (2.0)
Greece (*52*)	0/3016 (0.0)
Southern Italy (*130*)	6/361[a] (1.7)
	4/361[b] (1.1)
Northern Italy (*183*)	9/948 (0.9)
Netherlands (*180*)	5/1275 (0.4)
South Africa (*129*)	11/483 (2.3)
Spain (*113*)	25/863 (2.9)
Switzerland (*102*)	3/94 (3.2)
California—United States (*110*)	59/5,000[c] (1.2)
	70/5,000[d] (1.4)
Iowa—United States (*88*)	11/332 (3.3; 1989 data)
	0/111 (0.0; 1998 data)
Wisconsin—United States (*53*)	8/386[a] (2.1)
	9/386[b] (2.3)
Nonindustrialized countries	
Bolivia (*96*)	93/574 (16.2)
Chile (*80*)	17/237 (7.2)
China (*115*)	17/31 (54.8)
Egypt (*129*)	25/102 (24.5)
India (*15*)	37/200 (18.5)
Saudi Arabia (*129*)	82/861 (9.5)
Saudi Arabia (*1*)	72/488 (14.8)
Thailand (*132*)	28/178 (15.7)

[a] Determined by ELISA against recombinant HEV antigens.
[b] Determined by ELISA against synthetic HEV peptides.
[c] Determined by enzyme immunoassay against HEV-specific artificial mosaic protein.
[d] Determined by enzyme immunoassay against HEV recombinant protein.

atitis E infections in these countries indicates that there is a low level of HEV circulating in the environment.

POPULATIONS AT RISK

Race/geography. There is no evidence that race is a factor for HEV infection *(108)*; however, there definitely is a geographical bias. Hepatitis E is more prevalent in tropical and subtropical countries deficient in adequate socioeconomic and hygienic conditions. Epidemics of hepatitis E have occurred in Asia (Burma, China, the Central Asian Republics of the former Soviet Union, India, Indonesia, Nepal, and Pakistan), Africa (Algeria, Chad, Ethiopia, Ghana, Ivory Coast, Morocco, Senegal, Somalia, and Sudan), and Mexico. Outbreaks have not been reported from Latin America, except for Mexico, nor from industrialized areas such as Australia, Canada, Europe, Japan, New Zealand, and the United States *(5, 22, 30, 101, 108)*. Reports on anti-HEV IgG prevalence in Latin American countries indicate that HEV is endemic in Bolivia *(25, 96)*, Chile *(80)*, Cuba *(108)*, and Mexico *(7)*. It is significant that 1.7% (10 of 574) of blood donors tested in Bolivia were positive for anti-HEV IgM, which is indicative of recent infection *(96)*.

Age. During epidemics, the highest attack rate leading to clinically apparent hepatitis E is seen in individuals between 15 to 40 years of age, but clinical disease is infrequent in children *(5, 101)*. Khuroo and Kamili *(92)* described an epidemic of hepatitis E in India in which the infection rate, both clinical and inapparent infection, in children was 7.5% compared to 20% in adults. Clinical hepatitis was rarely seen in children <14 years of age. Dilawari et al. *(55)*, describing a waterborne epidemic of hepatitis E in India, noted that most children under 10 years of age were spared, and the highest rate of hepatitis cases occurred in individuals between 20 and 30 years of age. Arankalle et al. *(17)* and Hau et al. *(67)* demonstrated that the peak incidence of infection, as measured by the presence of anti-HEV IgG, was around age 20 to 40. However, in contrast to epidemic hepatitis E, sporadic hepatitis E has a similar prevalence in both adults and children *(20)*.

Young children are not often infected, nor do they show clinical signs of infection, during hepatitis E epidemics. Because HEV is a fecally-orally transmitted virus, children would be expected to be infected more frequently than adults. In many developing countries, HAV, also transmitted by the fecal-oral route, infects >90% of children by the time they are 6 years old with most cases being asymptomatic *(50)*. Arankalle et al. *(17)*, studying a population in Pune, India, found that 88.8% (168 of 189) of children aged 7 months to 10 years were positive for anti-HAV IgG, whereas only 5.2% (10 of 192) were positive for anti-HEV IgG. Similarly, Hau et al. *(67)*, studying a population in the Mekong River Delta region of Vietnam, found that 4.3% (4 of 92) of children aged 0 to 9 years were positive for anti-HEV IgG, but 83.7% (77 of 92) were positive for anti-HAV IgG. Thus, unlike HAV, prevalence of HEV (anti-HEV IgG) is low in children and increases with age *(17, 43, 67)*. Children are not immune to HEV, but infection and/or disease is infrequent. Nonetheless, acute hepatitis due to HEV infection does occur in children *(19, 20, 57, 78, 79, 106)*.

Sex. Males infected with HEV are more likely to have clinical hepatitis than females *(22, 108)*. However, depending on the study, the male-to-female ratio can range from 1:1 to 3:1 *(108)*. There is no sex bias in children with clinical hepatitis E *(22)*. While it is not clear why adult males are more susceptible to hepatitis E than women, the lack of a sex bias in children suggests that the occupational and societal roles that men perform may be responsible.

Pregnant women. Normal pregnancy leads to some level of liver dysfunction. Laboratory tests for hepatic function during pregnancy show changes that would be interpreted as incipient liver disease in nonpregnant patients *(51, 123)*. The most common cause of jaundice or serious liver disease during pregnancy is infection by hepatic viruses *(128)*. Most patients infected by HEV show a self-limited disease; however, fulminant hepatic failure (FHF) is often associated with hepatitis E, particularly during pregnancy *(109, 112)*. FHF, especially during the third trimester, can lead to increased fetal and maternal mortality *(55, 109, 120, 122)*. FHF is defined as acute liver disease associated with disturbance of neurological functions (i.e., hepatic-associated encephalopathy) and failure of key liver functions *(9)*.

In an early study of viral hepatitis during a waterborne epidemic in Kashmir, India, Khuroo et al. *(94)* determined that hepatitis E was more prevalent and led to higher mortality in pregnant women. More recent data also indicate that hepatitis E infections of pregnant women are more severe than in other segments of the population. In a waterborne epidemic occurring in Pakistan, Rab et al. *(140)* reported that the attack rate of hepatitis E in pregnant women was 21.6% (35 of 162). The attack rate in nonpregnant women of childbearing age and in men was approximately 10%. Death in infected pregnant women was 11.4% (4 of 35); all of the deaths occurred during the third trimester and were the result of FHF. Four newborns delivered of mothers with acute icteric hepatitis died during the epidemic *(140)*. Dilawari et al. *(55)* found that 19 pregnant women developed hepatitis during a waterborne epidemic of hepatitis E in India. Three of the 19 women developed FHF in the third trimester and died. Two other women aborted during the illness, and one had a preterm delivery *(55)*.

According to Mast and Krawczynski *(109)*, the overall case fatality rate for hepatitis E worldwide ranges from 0.5 to 3.0%. The hepatitis E case fatality rate during pregnancy ranged from 11.4 to 21.0% with a mean of 15.2% *(10, 26, 46, 55, 86, 94, 124, 140)*. Thus, hepatitis E during pregnancy is a serious health risk: pregnant women are more likely to be infected by the virus, more likely to develop FHF, and more likely to die with concomitant loss of the fetus. Reports indicate that abortion, death of the fetus in utero, premature delivery, or death of the baby soon after birth are seen in women with icteric hepatitis or with FHF induced by hepatitis E *(55, 94, 140, 170)*.

Transplacental transmission of HEV has received inadequate attention. A limited study involving eight babies born from mothers infected with HEV during the third tri-

mester demonstrated that six of eight babies showed evidence of hepatic infection at birth *(93)*. However, Velázquez et al. *(172)* found no evidence of transplacental transmission of HEV. Thus, it is not clear if intrauterine HEV infection of the fetus is common.

Why are pregnant women more susceptible to severe disease when infected with HEV? There are physiological and/or hormonal changes that occur during pregnancy that might play a role in the severity of HEV in pregnant women but there are no supporting data. Pastorek *(128)* suggested that many women in developing countries are lacking in medical and nutritional advantages available in industrialized countries. The lack of nutrition and medical care could account for the high maternal morbidity and mortality seen in hepatitis E. The immune system in women already immunocompromised by pregnancy could be further weakened by malaria and other parasitic infections, as well as by viral and bacterial infections common in developing nations; the increased immunocompromised state may increase susceptibility to infection by HEV. In addition, the medical infrastructure in developing nations, particularly in rural areas, is not adequate to care properly for pregnant patients with FHF, and as a result, death in these patients is frequent. Hussaini et al. *(77)* stated that intensive supportive care in a specialized liver unit is extremely important in preventing deaths due to acute liver failure. In addition, good perinatal care is needed to ensure survival of infants born to women with severe viral hepatitis.

ANIMALS AND HEV

Experimental models. Several animal species have been experimentally infected with human strains of HEV including nonhuman primates such as African green monkeys, chimpanzees, cynomolgus masques, owl monkeys, rhesus monkeys, and tamarins *(30, 98, 157)*; swine *(23, 117)*; noninbred white mice *(87)* and Wistar rats *(107)*. Animal experiments have been useful in demonstrating several facets of HEV infection. Inoculation of cynomologous monkeys with an HEV strain from Pakistan led to viremia and fecal virus excretion. The viral titer in serum was approximately 1,000-fold lower than that in feces. The titer of HEV RNA ranged from 10^3 to 10^4/ml in pooled sera and from 10^6 to 10^8/g in pooled feces *(165, 167)*. Following viremia, the levels of the liver enzymes, alanine aminotransferase (ALT), and isocitric dehydrogenase increased that was then followed by seroconversion. The immune response in the monkeys led to the end of viremia and fecal shedding with eventual resolution of the disease within 6 to 7 weeks after inoculation *(165, 167)*.

Tsarev et al. *(168)* inoculated pregnant and nonpregnant female rhesus monkeys with HEV and found that biochemical, histological, and serological parameters were similar in both sets of animals. There was no evidence of severe hepatitis in pregnant monkeys, and there was no transmission of virus to offspring. Thus, while rhesus monkeys are a useful animal model for hepatitis E, the animals did not show increased morbidity and mortality during pregnancy.

Rhesus monkeys infected with one Indian isolate of HEV were found to be immune 1.5 to 2.75 years after infection when challenged with four other closely related Indian strains of the virus *(14)*. There was no evidence of multiplication of HEV in the challenged monkeys. Long-term serological follow-up of HEV immunity in experimentally infected rhesus monkeys indicated that while the IgG anti-HEV titers could decline to low levels, animals were still protected against homologous or heterologous HEV challenge even 5 years after initial infection *(13)*. Thus, it appears that even low levels of HEV antibodies in animals are protective against infection.

Utilizing animals, Tsarev et al. *(167)* demonstrated the efficacy of vaccination against HEV. Control rhesus monkeys inoculated with the Pakistan strain of HEV demonstrated increased levels of ALT, histopathology consistent with hepatitis, viremia, and fecal shedding. Animals vaccinated with a recombinant vaccine (baculovirus-expressed 55-kDa ORF2 protein from the Pakistan strain) developed high titers (1:10,000) of anti-HEV antibody that protected them from the effects of HEV when challenged with the homologous Pakistan HEV strain. These animals did not show elevated ALT, and there was little or no liver histopathology. While diminished as compared to nonvaccinated animals, viremia and fecal shedding of virus was evident in most vaccinated animals *(167)*. Challenge of the vaccinated monkeys with the heterologous Mexico strain (Mex-14) of HEV demonstrated that the animals did not have elevated ALT or liver pathology, but viremia and fecal shedding of the Mexico strain were observed *(167)*. Strain Mex-14 demonstrates approximately 80% nucleotide and 90% amino acid homology with the Pakistan strain *(118)*. Cynomolgus monkeys, passively immunized with late convalescent plasma from a cynomolgus monkey with high-titer antibody against Chinese strains of HEV, were protected against hepatitis E when challenged with the Pakistan HEV strain *(166)*.

Thus, animal experiments indicated that HEV infection leads to the production of protective antibody against both homologous and heterologous HEV strains and that the protective effect lasts for at least 5 years. Vaccination with a recombinant HEV vaccine led to high titers of antibody and there was no increase in the levels of hepatic enzymes and no evidence of hepatic histopathology. Vaccination by one strain protected against clinical disease by another strain of the virus. However, vaccination did not prevent infection or preclude fecal shedding of the virus and viremia. Passive immunization also protects animals against hepatitis E.

Oral inoculation of monkeys with HEV is not a very efficient way to induce disease. Using a stool specimen from a Pakistani patient standardized to contain 10^6 cynomolgus 50% infection doses of HEV/g of feces, Tsarev et al. *(169)* found that a 10^{-1} dilution of the standard suspension, when given orally, did not produce disease in monkeys, whereas dilutions ranging from 10^{-1} to 10^{-5} of the stool suspension given intravenously were infective. However, it is probable that intravenous inoculation elicits an immunological response quite different from that of the natural oral route of infection seen in humans. An efficient system

of replicating the virus must be developed before studies on oral infection of animals can be done.

Potential animal reservoirs. There is evidence that animals may act as natural hosts of HEV. Wild-caught rodents *(41, 60, 84, 87)*; wild-caught old world monkeys such as rhesus, bonnet, or langur monkeys *(16, 24, 168)*; and wild-caught new world monkeys such as marmosets and tamarins *(31, 86)* were positive for anti-HEV antibodies and/or presence of viruses in sera or stools. However, it is not clear if the animals had been infected by animal or human viruses. Fecal excretion of HEV has been demonstrated in rats *(107)*, mice *(87)*, and monkeys *(167)* experimentally inoculated with human HEV strains. It is probable that animals can be infected in nature by HEV from human feces, and infected animals can, in turn, infect humans by fecal contamination of water and foodstuffs.

What is more interesting and also more disturbing is the demonstration of HEV in certain domestic animals. Anti-HEV has been detected in domestic cattle from Turkmenistan (Central Asia) and Ukraine *(61)*. Hartmann et al. *(66)* found that 74% of 50 pigs at slaughter in Bavaria were seropositive for HEV. IgG antibodies against HEV were found in 13.6% (9 of 66) of domestic chickens and in 28.8% (30 of 104) of domestic swine from the Kathmandu Valley in Nepal; cows, goats, and water buffaloes were negative for HEV IgG *(44)*. HEV RNA was also present in the feces and sera of pigs from the Kathmandu Valley *(41, 44)*. Balayan et al. *(23)* infected swine with a human strain of HEV and demonstrated elevated ALT and jaundice of skin and sclera, as well as the presence of fecal virus particles that reacted with antibody from HEV-infected patients. Pigs inoculated with antibody-neutralized virus did not develop symptoms *(23)*. Similarly, Meng et al. *(117)* demonstrated seroconversion and fecal shedding of HEV in pigs inoculated with a human strain of the virus. Because swine can be infected with human strains of HEV and because human HEV RNA has been detected in swine sera and feces, it is highly possible that swine could contaminate water supplies with the virus and that transmission of virus could occur during slaughtering operations. Raw pork from pigs infected with human HEV could lead to cross-contamination of other foods, and inadequately cooked pork could be a vehicle for foodborne HEV.

HEV in U.S. wild rats. When trapped wild rats in Baltimore, urban and rural areas of the islands of Oahu and Hawaii (Hawaii), and New Orleans were tested for anti-HEV IgG, seropositivity in the rats was 77.1% (64 of 88), 44.4% (4 of 9), and 89.8% (132 of 147), respectively *(84)*. Overall, 83.7% (200 of 239) of the rats were seropositive for the HEV capsid protein. Hepatitis E virus RNA was not detected in the sera from any of the seropositive rats *(84)*. Favorov et al. *(60)* demonstrated that 33% of 806 rodents (26 species from 15 genera) trapped in the United States were HEV seropositive. The highest incidence of HEV seropositivity was 59.7% (166 of 278) in the genus *Rattus*. Rodents from urban areas had a higher incidence of seropositivity than animals trapped in rural areas *(60)*. Because virus was not detected in sera of the rats, the genotype of the virus could not be determined. These data indicated that HEV is widespread in rodents in the United States, and it is possible that rodents may be reservoirs for HEV. The possibility of food and water contamination by feces from rodents infected by HEV should not be discounted.

TABLE 2. *Incidence of anti-HEV IgG in pigs*

Country	Incidence of anti-HEV IgG in pigs: No. positive/ total no.	Incidence of anti-HEV IgG in pigs: % positive	Reference
Australia			
Commercial pigs	12/40	30.0	*36*
Wild pigs	15/59	25.4	
Canada			
Quebec	116/400	29.0	
Ontario			*115*
Total	13/312	4.1	
Adult pigs[a]	12/82	14.6	
Nursery pigs[b]	1/230	<1	
China	22/72	30.6	*115*
Germany			
Bavaria (at slaughter)	37/50	74.0	*66*
Korea	57/140	40.7	*115*
Nepal			
Kathmandu Valley	48/159	30.2	*41, 44*
Taiwan	102/275	37.0	*73*
Thailand	23/75	30.7	*115*
United States			
Midwest	192/243	79.0	*115*

[a] Pigs were 8 months to 3 years old.
[b] Pigs were approximately 8 weeks old.

Swine HEV. The incidence of anti-HEV IgG in pigs from various parts of the world is given in Table 2. The incidence ranged from 4.1% in Ontario, Canada to 79.0% in the midwestern United States. The data presented in Table 2 indicate that anti-HEV IgG is present in pigs from HEV endemic countries such as China, Nepal, and Thailand as well as from pigs in nonendemic countries such as Australia, Canada, Germany, Korea, Taiwan, and the United States. Thus, HEV is enzoonotic in pigs even though the virus may or may not be endemic in the resident human population.

Meng et al. *(118)* identified a viral strain in U.S. swine that cross-reacted with antibody to the capsid antigen (ORF2) of human HEV. The virus was designated as swine hepatitis E virus (swine HEV) and was present in 192 of 243 (79%) of pigs ≥2 months of age from herds in the midwestern United States. Naturally infected young pigs developed viremia before seroconversion. These piglets demonstrated microscopic evidence of a mild hepatitis, but there was no increase in liver enzyme activity nor jaundice; thus, the pigs were clinically normal.

Swine HEV from the United States is closely related, antigenically and genetically, to strains of human HEV. Se-

quencing of U.S. swine HEV ORF2 region indicated a genetically distinct strain that had a close phylogenetic relationship to the Asia-Africa and Mexico genotypes of HEV with 79 to 80% nucleotide homology and 90 to 92% amino acid homology *(118)*. The greatest degree of sequence similarity was to the HEV strains US-1 and US-2 from the United States with 92% nucleotide homology and 98 to 99% amino acid homology for ORF2 *(117)*. Due to the high degree of similarity, swine HEV (from midwestern U.S. pigs) is included in the U.S. genotype.

In further studies, Meng et al. *(116)* injected specific-pathogen-free pigs with swine HEV. Viral RNA was demonstrated in sera, feces, and nares of the infected animals. However, the animals were clinically normal in spite of infection. The presence of HEV in the nares would indicate that infection could take place via rooting in manure contaminated with swine HEV *(116)*. Injection of specific-pathogen-free pigs with human HEV strains from Burma and Mexico did not lead to infection even at 1,000 to 10,000 times the level of the swine HEV inoculum necessary to produce infection in pigs *(116)*. However, intravenous inoculation of specific-pathogen-free pigs with the US-2 strain of human HEV did lead to infection of the pigs as shown by the presence of US-2 viral RNA in pig feces *(117)*. The US-2 strain shows a higher nucleotide and amino acid homology to swine HEV than do the Burma and Mexico strains.

When rhesus monkeys were inoculated intravenously with swine HEV, the monkeys showed seroconversion, fecal shedding of virus, and viremia. There was mild acute hepatitis with a slight elevation of liver enzyme activity *(117)*. A chimpanzee injected with swine HEV seroconverted and shed virus in feces but did not show viremia or signs of hepatitis. Viruses from the feces of monkeys and the chimpanzee were sequenced and confirmed as swine HEV *(117)*. The fact that nonhuman primates could be infected by swine HEV strongly suggests that swine HEV could infect humans; however, it is not certain that humans would show signs of clinical hepatitis. The close relationship among US-1, US-2, and U.S. swine HEV indicates that swine may be a (or the) reservoir for HEV in the United States and in other parts of the world.

A survey of pigs from 10 herds in Taiwan indicated that 37.1% (102 of 275) were positive for HEV IgG *(73)*. One pig was positive by RT-PCR for HEV RNA. Using pairwise comparison based on a 185-bp sequence within ORF2, the Taiwan swine virus showed 97.3% nucleotide sequence identity with human Taiwan HEV, but only 70 to 77% nucleotide identity with human HEV strains of the Asia-Africa, Mexico, or the U.S. genotypes. The Taiwan swine virus demonstrated only 80% identity with U.S. swine HEV *(73)*. Recently, Wu et al. *(176)* showed that HEV strains present in Taiwanese swine from herds not studied by Hsieh et al. *(73)* were closely related genetically to virus found in hepatitis patients in Taiwan, but not closely related to HEV from other genotypes. Thus, the work of Wu et al. *(176)* corroborates the study of Hsieh et al. *(73)*.

Epidemics of hepatitis E have not occurred in Taiwan, but more than 10% of sporadic non-A, non-B, and non-C hepatitis cases are due to hepatitis E *(74)*. The origin of these sporadic cases in Taiwan is unknown; however, travel by the affected individuals to HEV endemic areas was not an explanation. The finding that Taiwan swine HEV is closely related to the Taiwan human HEV would suggest that sporadic cases of HEV seen in Taiwan involve transmission of the virus from swine to humans. Individuals who worked for pork companies but had no direct contact with pigs were twice as likely to have anti-HEV IgG as compared to controls. Pig handlers were three times more likely to be HEV seropositive than controls *(73)*.

The preliminary work by Meng et al. *(117)* in the United States and Hsieh et al. *(73)* in Taiwan suggests that the swine and human HEV strains found in a particular geographic region are closely related genetically. However, more studies are needed to determine if there is a close relationship among swine and human HEV for specific geographic regions. Such studies should not be limited to swine but should include other domestic and wild animals because there is a wide prevalence of HEV antibodies in animals.

The presence of HEV in swine herds indicates that run-off, such as rain water that has not drained into the soil, from pig farms could contaminate irrigation and surface waters. Run-off water from swine farms could be a source of HEV contamination for coastal shellfish beds located close to those farms. Viral hepatitis due to the ingestion of hepatitis A virus in shellfish has occurred frequently *(141)*. Thus, if HEV present in run-off waters is allowed to enter coastal water environments, the virus would possibly accumulate in shellfish *(32)*. Thus, in terms of public health, swine HEV is a zoonosis with implications for human illness and may prove to be a xenozoonosis if xenotransplantation of pig tissues and organs becomes a common medical practice.

The close genetic and serological relationship of swine HEV to various strains of human HEV indicates that the swine virus would be an ideal agent for the development of vaccines against HEV and that the virus could be used to develop diagnostic tools for HEV detection. Swine HEV also could be used as a model to learn more about the biology of the virus. In comparison to nonhuman primates, swine are relatively inexpensive to house, feed, maintain, and breed.

MANAGEMENT, CONTROL, AND PREVENTION OF HEV

Management of pregnant patients. Pregnant women are at high risk for more severe disease when infected by HEV, and their care during the course of the illness must be closely supervised. Care of the pregnant patient with hepatitis E is largely supportive. Less severely ill women can be treated as outpatients. They need to reduce their level of activity, avoid upper abdominal trauma, and maintain good nutrition *(12)*. However, the infected pregnant patient should be hospitalized if there are signs of encephalopathy (i.e., neurological symptoms), coagulopathy (i.e., blood coagulation disease symptoms), or severe debilitation. Nutrition, fluid, and electrolyte needs must be addressed. In cases where there is presentation of coagulo-

pathy, fresh frozen plasma should be administered *(12)*. The production of ammonia plays a major role in encephalopathy of acute liver failure. Treatment with lactulose or neomycin can be used to reduce ammonia production by gut microorganisms *(68)*. Depending on the severity of their symptoms, the same type of support as described above should be given to male and nonpregnant female patients infected with HEV *(12)*.

Control. In epidemic areas, HEV is transmitted mainly by fecally contaminated water. Improvement of water quality by proper sewage disposal and water treatment is an important preventive means. When there are failures of sanitation and hygienic facilities, the boiling of water used for drinking, cooking, and bathing is effective in preventing transmission of the virus. Neither raw nor cooked foods should come in contact with HEV-contaminated water, and HEV-infected individuals should not be allowed to prepare or handle foods for others *(50)*. Education of individuals in epidemic and endemic areas regarding proper personal and food/water hygiene should aid in the control of hepatitis E.

Travelers to hepatitis E endemic areas must take special precautions. The traveler must practice good personal hygiene, boil or otherwise disinfect drinking water, avoid ice cubes, avoid uncooked shellfish and only eat thoroughly cooked foods. If raw vegetables or fruit are to be eaten, those that cannot be peeled should be avoided *(101)*.

Vaccines. Vaccination against HEV would protect individuals and pregnant women against epidemic and sporadic infections in countries endemic for HEV. Vaccines also would protect travelers who visit hepatitis E endemic countries *(178)*. Unfortunately, there is currently no vaccine available against HEV. The ORF2 protein product should be an effective immunogen for the generation of protective antibodies against HEV infection. Nonhuman primates, injected intramuscularly with a recombinant antigen expressed by HEV ORF2, seroconverted to anti-HEV status *(167)*. When vaccinated animals were challenged intravenously with either homologous or heterologous strains of HEV, they were protected against hepatitis but not against infection *(138, 166, 167)*. It should be noted that animals were challenged by intravenous inoculation and not by the normal oral route. The animal vaccination experiments suggest that humans would be protected against HEV by use of recombinant vaccines.

Vaccines can be potentially useful in generating humoral and cellular immune responses. Hepatitis E virus DNA encoding the ORF2 protein should prove to be an effective immunogen *(65)*. He et al. *(69, 70)* have studied immunity against HEV by the use of DNA vaccination. High titers of anti-HEV IgG were elicited by the intramuscular injection of mice with a plasmid construct containing the complete DNA sequence of HEV ORF2. Antiserum from the DNA-immunized mice reacted specifically with the structural protein expressed by ORF2 *(70)* and formed aggregates, visualized by immunoelectron microscopy, of the virus present in bile from an HEV-infected monkey *(69)*. In addition, the antiserum was used in the immunocapture of virus from patients' stools for amplification in RT-PCR *(69)*. The work of He and coworkers *(69, 70)* suggests that DNA-based immunization by HEV ORF-2 DNA is feasible.

ECONOMICS OF HEV INFECTION

Morbidity, disability, and costs associated with HEV-induced disease can be substantial. A recent study from Nepal indicated that the median yearly income loss for a wage earner with hepatitis E was 19.4% *(40)*. The infected individuals were ill for a median of 22 days and bedridden for 10 days. The median number of days lost due to illness (i.e., bedridden, sick but not bedridden, and not sick but with residual disability) by an individual was 35 days. The data presented by Clark et al. *(40)* indicate that in hepatitis E endemic countries, disease caused by the virus places a heavy economic burden on developing countries in terms of monetary loss and loss of productivity.

FINAL PERSPECTIVES

In industrialized countries, HEV infection from properly treated drinking water would be unlikely. It is more probable that vegetables and fruit might be the source of HEV transmission, especially if those products are obtained from underdeveloped countries. There has been a 21.6% increase in the consumption of fresh fruit and vegetables by U.S. consumers since 1970 *(139)*. These products are being demanded year-round, and as a consequence, such foods are increasingly obtained from a global market. More than 75% of fresh produce eaten in the United States is harvested and shipped from Central America, Mexico, and other tropical areas *(72)*. Some of the countries sending vegetables and fruit to the United States are endemic for HEV. For example, assay of serum samples of individuals from various parts of Mexico indicated that 10.5% (374 of 3,549) were anti-HEV positive *(7)*. A seropositivity rate of 10.5% indicates that HEV is endemic in Mexico.

In hepatitis E endemic areas, crops irrigated with fecally contaminated water could lead to the contamination of produce with enteric pathogens. Surface waters used for irrigation may be a source of HEV, if such waters are contaminated by raw sewage, by improperly treated effluents from sewage plants, or by run-off from farm manure piles, animal waste lagoons, or animal pens. Run-off from crop lands fertilized by raw or improperly treated sewage sludge or animal manures may contaminate irrigation water *(152)*. The preliminary work of Hsieh et al. *(73)* and Meng et al. *(117)* suggesting that swine may be a reservoir of the virus indicates that swine manure could be an important source of HEV contamination for irrigation water. In addition, if irrigation water is used as drinking water by wild and/or domestic animals, there is a possibility that the water will be fecally contaminated by the animals. Other potential sources of fruit and vegetable contamination include poor worker hygiene, washing of produce with fecally contaminated water, and transportation of fruit and vegetables under insanitary conditions.

There is the possibility that HEV will be introduced into developed nations from vegetables, fruit, or other foods imported from hepatitis E endemic countries. Public health

systems must take proper precautions and be prepared to cope with a potential increase in hepatitis E.

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