BLOOD PRODUCTS ADVISORY COMMITTEE
104th Meeting, September 20, 2012
FDA Fishers Lane Building
5630 Fishers Lane, Room 1066

Rockville, MD

Issue Summary

Topic I: Hepatitis E Virus (HEV) and Blood Transfusion Safety

Issue: FDA is seeking advice from the Blood Products Advisory Committee whether the available scientific data indicate a need to determine the risk of HEV transmission by transfusion in the U.S., and, if so, a) what studies would be most useful to address this risk; and b) how best to characterize HEV assays for use in such studies.

Introduction:

Hepatitis E virus (HEV) has been recognized since 2004 as a transfusion transmissible infectious agent and recent epidemiological data suggest that it may pose a safety threat to the U.S. blood supply. Although there have been no cases of transfusion transmitted infection reported in the U.S., there are documented cases from Japan, the United Kingdom, Saudi Arabia and France.[1-7] Although documented cases of HEV infection are uncommon in the U.S., some studies have detected a high prevalence of antibodies to HEV in blood donors suggesting the possibility of transmission by blood transfusion.[8-10] HEV generally causes a self-limiting illness but may cause acute fulminant hepatitis in the settings of pregnancy, underlying liver disease and immune compromise.[11-15] Recently it was reported that worldwide HEV infection causes >3 million symptomatic cases of acute hepatitis E each year that result in approximately 70,000 deaths.[16] CDC reports that the ratio of symptomatic to asymptomatic cases ranges from 1:2 to 1:13 in the developing world.[17] Chronic infections are rare, and have been seen in solid organ transplant recipients.[18-24] Persistent infection may also occur in individuals with compromised immune systems including cancer patients undergoing chemotherapy, HIV-infected patients, and individuals with drug induced liver failure and liver damage due to alcohol abuse.[25-29] Chronic HEV infection may progress to cirrhosis or death.[18,21]

The incidence of HEV in the general U.S. population is unclear and likely underestimated due to the lack of well validated assays, asymptomatic infections and the fact that HEV is not a reportable disease. Estimates of seroprevalence reported in the U.S. are also variable and thought to depend largely on the different performance characteristics of the assays used in the various studies. Limited studies in blood donors have suggested significant seroprevalence, but incidence in donors is not well characterized. Based on the available information, the Committee will be asked to discuss the potential risk of HEV to transfusion safety. Comments will be requested on a phased approach to address HEV risk to transfusion including development and use of pedigreed panels and reagent

standards, validation of nucleic acid tests (NAT) and serological assays, studies to determine the prevalence of HEV NAT-positivity in blood donors, and studies of transmission of HEV by transfusion.

Background:

HEV is an important cause of acute hepatitis worldwide. Although it is believed to be uncommon in the U.S. and other developed countries, HEV is responsible for >25% of non-A, non-B hepatitis in developing countries (Figure 1). The disease is endemic in many developing countries in Southeast and Central Asia, the Middle East, Central America and Africa, primarily due to contaminated drinking water. Sporadic cases of acute HEV have been observed in developed countries throughout the world such as in Europe and the U.S., where it was originally thought to be due to travel to endemic areas, but more recently has been found in individuals with no travel history. Some of these infections have been determined to be caused by food-borne transmission from a zoonotic reservoir, most often swine, and rarely by blood transfusion or organ transplantation.[30]

HEV was discovered after a large epidemic of acute viral hepatitis that caused ~29,000 cases that occurred in 1955-56 in New Delhi, India, following severe flooding that led to contamination of drinking water with sewage. The outbreak was initially thought to be caused by hepatitis A virus (HAV) because that was the only hepatitis virus recognized as waterborne at that time. Fifteen years later, sera from those infected in the Delhi epidemic and two subsequent outbreaks in India (Ahmedabad and Pune) were tested. None of these patient samples were determined to have evidence of HAV infection and only 1% had evidence of HBV infection. Therefore, it was determined that the outbreaks were caused by an unknown enteric non-A, non-B hepatitis agent. This new agent was recognized in 1980 but the virus was not visualized until 1983 using immune electron microscopy of feces.[31-33] After the genome was cloned and sequenced in 1991, the virus became known as hepatitis E virus.[34] In a recent publication by Kamar, *et al*. the discovery of HEV was further substantiated by the outbreak of unexplained hepatitis in soldiers in Afghanistan during the Soviet occupation in the 1980s, and the HEV was detected by electron microscopy in the stool of a person who ingested fecal material with yogurt. [35]

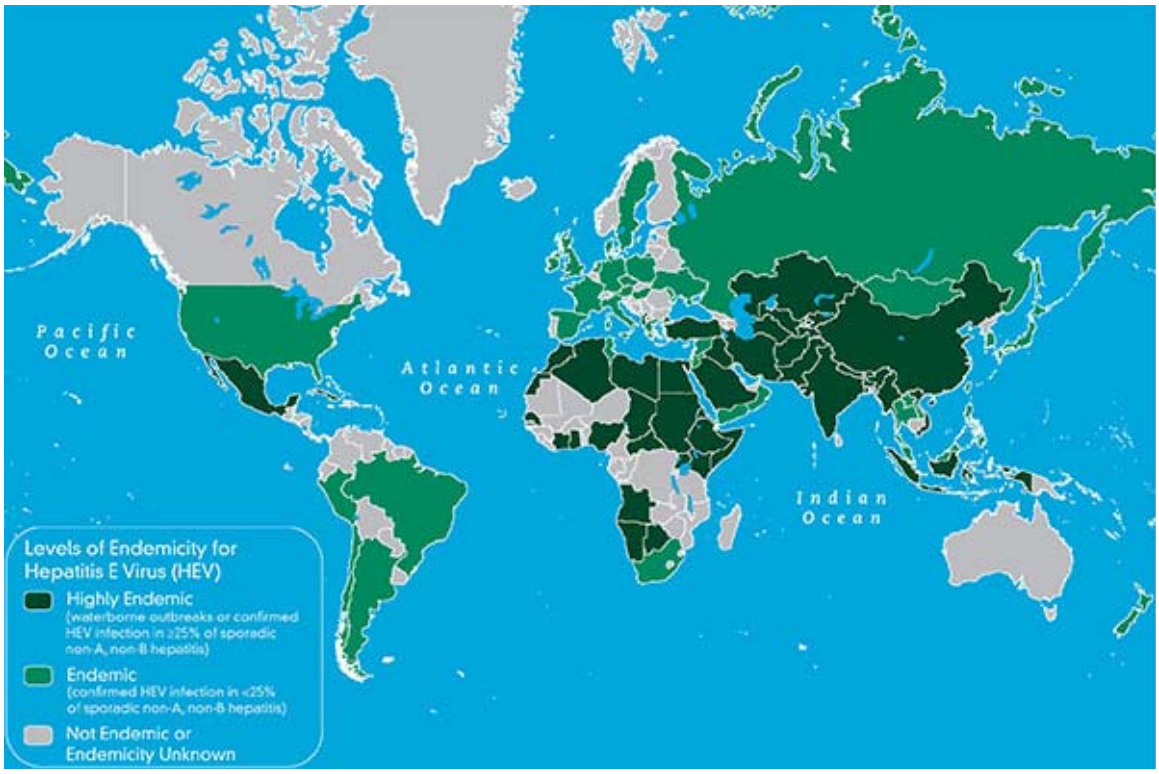

Figure 1: Geographic Distribution of Hepatitis E Infection (2010)
(Teo, Chong-Gee, Centers for Disease Control and Prevention, 2012 Yellow Book)

HEV is a small, nonenveloped, RNA virus belonging to the *Hepevirus* genus in the *Hepeviridae* family. The virion is 27-34 nm in diameter with a positive-sense, single-stranded, 7.2 kb genome. There are four major genotypes (genotypes 1 to 4) representing one serotype causing infection in humans worldwide (Figure 2). HEV genotypes 1 and 2 only infect humans and are transmitted by contaminated water in developing countries. Epidemics in the developing world are primarily caused by genotype 1 in Asia and genotype 2 in Africa and Mexico. HEV infection in China is mainly caused by genotype 4, but genotype 1 and, recently, genotype 3 have been associated with the disease. Both HEV genotypes 3 and 4 infect humans, domestic swine and wild boar, deer and other animals and are responsible for sporadic cases of autochthonous HEV in both developing and developed countries. HEV genotype 3 is distributed worldwide and is the only genotype currently identified as the cause of autochthonous infection in the U.S. HEV genotype 4 is prevalent in eastern China, Taiwan and other Asian countries including Japan and has just been identified in France.[79] There have been no epidemics reported in industrialized countries.[35-36]

Although the main mode of HEV transmission is by the fecal-oral route through contaminated drinking water, foodborne transmission from consumption of undercooked or raw meat, organ meat and possibly shellfish, and zoonotic transmission by contact with infected swine or other domestic or wild animals has been documented in both developing and industrialized countries.[37-41] In one study, pig livers sold in local groceries stores in the U.S. were tested by HEV NAT. Of the 127 tested, 14 (11%) were positive for HEV RNA that was determined to be genotype 3. Uninfected pigs inoculated

with NAT-positive pig liver homogenate became infected, indicating long-term survival of the virus at retail food storage temperatures.[42]

In 1997 the first animal strain of HEV to be identified and characterized was swine HEV (now known as genotype 3) from pigs in the U.S. that was closely related to human HEV.[43] Pigs are the major HEV animal reservoir in the U.S. and can transmit genotype 3 to humans including pig farmers, veterinarians and workers in slaughter houses.[41] The extent of HEV-containing fecal waste from pig farming operations in the U.S is not known. Infectious HEV has been found in pig manure storage facilities in the U.S.[45] In North Carolina, HEV RNA was detected in stored swine liquid waste.[46] It is possible that some waste may contaminate the irrigation water of vegetables and other crops. Run-off into coastal waters may cause contamination of shellfish.

In 2008 acute HEV infection was confirmed in four passengers on a cruise ship returning to the United Kingdom after a world tour. Because they had been vaccinated for HAV, HEV was considered and then confirmed to be the cause. Sequencing of HEV RNA acquired from three of the four passengers determined the virus to be genotype 3, with homology to genotype 3 viruses from Europe. After a thorough investigation, the cause for the outbreak was determined to be foodborne from the shellfish consumption on the ship but the shellfish were not available for testing.[47]

In the U.S., HEV has been genetically identified in mammals, including domestic pigs, wild boar, deer, rabbits and wild rats (Table 1).[41, 43, 48, 80] However, HEV infection has also been reported in chickens and in fish.[50-51]

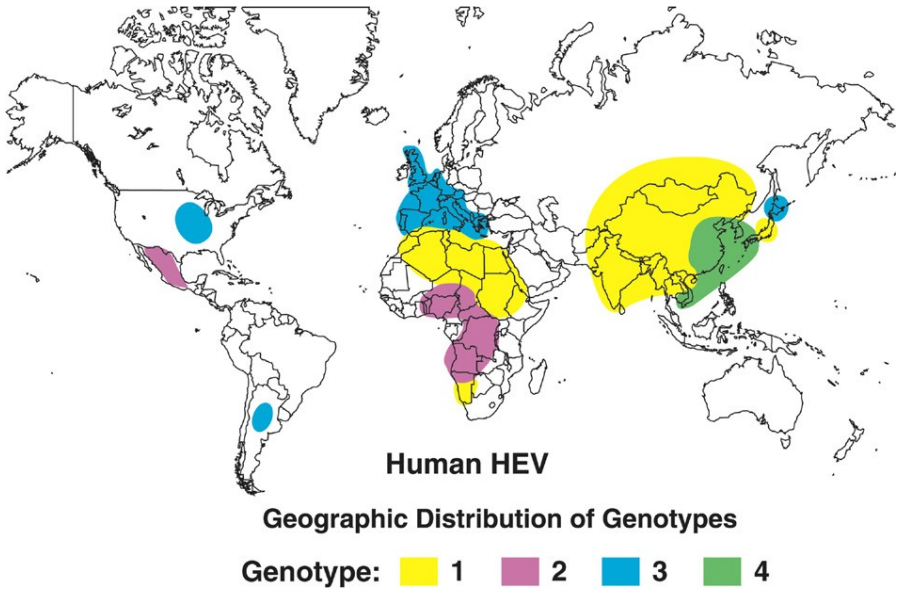

Figure 2: Geographic Distribution of Four Human HEV Genotypes
(Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. J Hepatol. 2008 Mar;48(3):494-503.)

HEV Strain	Natural Host	Experimental Host
Genus *Hepevirus*		
Genotype 1	Human	Non-human Primate, Rat, Lamb
Genotype 2	Human	Non-human Primate
Genotype 3	Human, Swine, Deer, Mongoose, Rabbit, Rats	Non-human Primate, Swine
Genotype 4	Human, Swine	Non-human Primate, Swine
Putative Genotype 5	Rats	
Putative Genotype 6	Wild Boar	
Putative Genus *Avihepevirus*		
Genotype 1	Chicken (Australia)	Turkey, Chicken
Genotype 2	Chicken (U.S.)	Turkey, Chicken
Genotype 3	Chicken (Europe and China)	Turkey, Chicken
Putative Genus *Piscihepevirus*		
Cutthroat Trout Virus	Fish	

Table 1: Proposed HEV Genotypes, Host Range and Cross-Species Transmission under Natural and Experimental Conditions
(Adapted from Meng, XJ Abstract from Hepatitis in the United States, An NIH Workshop, March 26, 2012)

HEV infection generally causes an acute, self-limiting hepatitis with symptoms very similar to those of other forms of viral hepatitis infection, particularly hepatitis A, with fatigue, jaundice, fever, malaise, nausea, vomiting, anorexia and abdominal pain. The incubation phase for HEV is generally 3-8 weeks, but longer and shorter periods have been reported. Acute viremic titers may reach up to 7 $\log_{10}$ RNA copies/mL. Viremia after infection lasts 4-6 weeks with RNA being detected in some cases for over 100 days. A longer duration of nucleic acid detection has been reported in immunocompromised organ transplant recipients following acute HEV.[23] Both anti-HEV IgM and IgG titers increase soon after infection in the asymptomatic phase. Anti-HEV IgM titers peak during the symptomatic phase and decline to baseline levels within 3-6 months after symptomatic disease. Anti-HEV IgG titers may remain detectable for up to 15 years[30, 35-36] (Figure 3)

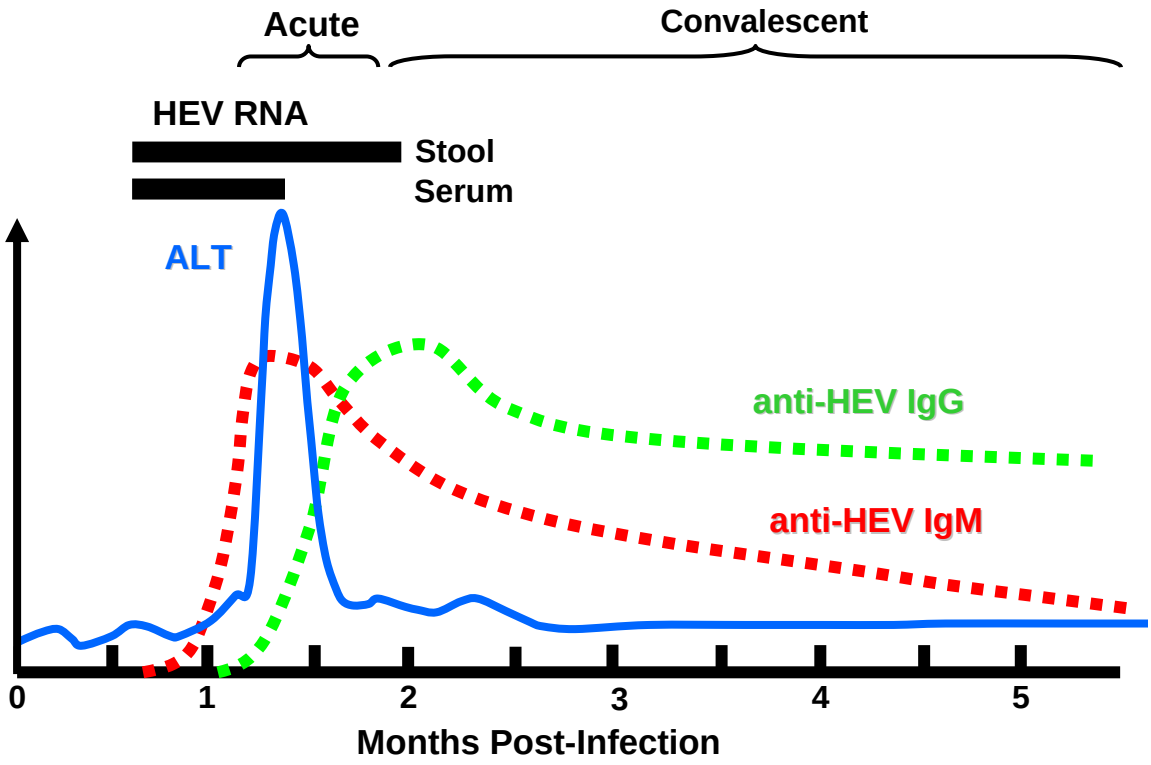

Figure 3: Schematic of Typical HEV Clinical and Serological Course Post-Exposure

Recovery from HEV infection occurs within 6-8 weeks. Many infections are mild or asymptomatic with mortality rates ranging from 0.2-4% in the general population in developing countries. Individuals with pre-existing liver disease, immunocompromised individuals including those on chemotherapy, HIV-infected individuals and pregnant women, especially in the third trimester may experience more severe clinical disease. Mortality rates of over 20% have been reported in pregnant women in developing countries.[12, 53] Most infections with HEV genotype 3 and 4 appear to be asymptomatic or undiagnosed, i.e., there are fewer clinically recognized symptomatic HEV infections associated with genotypes 3 and 4. Symptoms, when they occur, consist of icteric hepatitis, and are seen more often in older males, individuals with high alcohol consumption or, on occasion, drug-induced liver injury.[35] The absence of reports of fulminant HEV hepatitis in pregnant women in developed countries is unexplained, but may be related to lesser virulence of genotype 3 viruses in those regions or possibly better nutritional status.[53] Chronic infections have been observed mostly in solid organ donor recipients, but also in a few individuals with HIV infection and patients with hematological disorders receiving chemotherapy.[18-27,35] These infections can lead to end-stage liver disease, but this appears to be rare. There can also be neurologic manifestations associated with HEV, including Guillain Barré Syndrome.[18-24]

Treatment for acute or chronic HEV infection is generally supportive. Some patients with severe acute HEV infection have responded to ribavirin therapy but this treatment is contraindicated in pregnant women. Interferon-α and ribavirin therapy separately or in combination has been used for patients with chronic infection.[57-62]

A HEV vaccine has been developed by Chinese scientists that protects recipients up to three years from both infection (>70% efficacy) and disease (>90% efficacy).[63-64] This vaccine will be the first that is commercially available, reportedly by the end of the year; however, there are no FDA-licensed vaccines.

Discussion:

A. HEV Seroprevalence and Risk of Transfusion Transmission

1) Transfusion Transmission Worldwide:

HEV can be transmitted through transfusion. There have been several instances of post-transfusion HEV reported in developed countries including Japan, the United Kingdom and France, and Saudi Arabia. A detailed description of the reported cases of transfusion transmission of HEV infection is provided in **APPENDIX 1**.

2) Studies of HEV Incidence and Seroprevalence in the U.S.

In a single available study, the incidence of HEV was reported to be 0.7% per year in susceptible persons.[65] The actual incidence is unknown. Subclinical or undiagnosed cases of HEV infection and the fact that HEV is not a reportable disease in the U.S. complicate the estimate of incidence. The seroprevalence of anti-HEV in the general U.S. population and in special populations including blood donors is uncertain. Estimates of the U.S. seroprevalence range from less than 7% to 31% in various studies.[8-10, 66-67] This variation in reported seroprevalence likely reflects geographical and demographic differences of the populations tested, the different sensitivity and specificity of the assays used in the studies, high frequency of asymptomatic infections as well as other factors. Exposure of farmers, veterinarians and others in the pig industry to domestic pigs may play a role in seroprevalence especially in the Midwest. More than 80% of pigs in some U.S. herds are infected with HEV.[43] Additionally, consumption of undercooked pig meat or pig liver and wild boar meat may cause increased exposure to HEV. A description of recent and current studies to determine the incidence of HEV infection and the seroprevalence in the U.S. is presented in **APPENDIX 2**.

3) Risk of Transfusion Transmission in the U.S. is Unknown

There have been no cases of post-transfusion HEV reported in the U.S., and the risk of HEV infection from blood transfusion in the U.S. is unknown. Donated blood is not tested for HEV, and no commercially available, FDA-approved tests currently exist. Transfusion transmitted HEV infection may be occurring in the U.S. The high rate of subclinical infections and lack of diagnostic tests are confounding factors in determining the risk of transfusion transmission of HEV infection. This risk of acquiring HEV infection by blood transfusion likely varies according to the underlying clinical status of different recipient populations. Immunocompetent recipients are less likely to develop clinical disease from HEV-contaminated blood than immuno-suppressed patients,

pregnant women and those with underlying liver disease who may suffer increased morbidity and mortality.

Several studies to determine the prevalence of anti-HEV among U.S. blood donors have been reported. In one study, 300 blood donors from Baltimore, MD, 300 from New York City, NY and 211 from Sacramento, CA were tested for anti-HEV antibodies. Of the samples tested, 21.3%, 31.0% and 13.7% respectively were found to be positive for anti-HEV.[8] An ongoing study at the National Institutes of Health has found a comparable seroprevalence with 22.3% anti-HEV IgG positive and 0.3% anti-IgM positive after testing of over 1000 blood donors. Donations that were anti-HEV antibody positive were tested by NAT. None of them were HEV RNA positive. Prevalence of anti-HEV in pre-transfusion samples from 75 recipients was 22.7%. No seroconversion or HEV RNA has been detected after testing of such samples.[10]

In another study the IgG anti-HEV from 389 swine veterinarians from eight U.S. states (Minnesota, Indiana, Nebraska, Iowa, Illinois, Missouri, North Carolina and Alabama) was compared with 400 normal U.S. blood donors. Anti-HEV prevalence in swine veterinarians was determined to be 26% when assayed with a human HEV antigen and 23% with a swine HEV antigen. Only 18% of the blood donors from the same eight states were positive with the human HEV antigen and 17% with the swine antigen. These studies suggest that the swine veterinarians were more likely to be anti-HEV positive when tested with either a human or swine HEV antigen than normal blood donors.[9]. Therefore, these studies suggest that US blood donors are exposed to HEV which implies that there is a potential risk for transfusion transmission of HEV.

B. Consideration of Studies to Assess the Risk of HEV Exposure and Transmission by Transfusion in the U.S.

The apparent high seroprevalence of HEV in U.S. blood donors (13.7-31%) [8-10] has raised a potential concern for blood safety. However, the rate of seropositivity in donors does not provide an estimate for the rate of viremic donations, which are more likely to cause infectious exposures to recipients. An estimate of the viremic exposure rate could be obtained from NAT based studies in donors. A WHO International Standard for HEV RNA is available; [71] hence, sensitivity of candidate NAT assays can be benchmarked, and those assays used to study the risk of HEV transmission through blood transfusion based on rate of NAT positivity in blood donations.

An estimate of the exposure risk would not in itself establish the risk of virus, let alone disease transmission. In order to determine the risk of transfusion transmission of HEV in the U.S., large linked donor-recipient studies using both suitably validated NAT assays and suitably validated serology tests to show seroconversion in recipients would need to be performed. At the present time, a WHO International Reference Material for HEV serum IgG exists[72] that could be used to assess sensitivity of candidate assays for antibodies to HEV. However, a broader approach to characterization of HEV serologic assays based on performance with pedigreed clinical specimens would be needed to better characterize these assays.

A discussion of historic and ongoing studies to assess the performance characteristics of various NAT and serological assays is presented in **APPENDIX 3**.

FDA suggests that assessment of the transfusion risk from HEV could be approached in a phased set of studies, as follows:

1. Characterize NAT assays using the 1st WHO International Standard for HEV RNA and a panel of virologically confirmed clinical samples.

The 1st WHO International Standard for HEV RNA (using HEV genotype 3a) is suitable for benchmarking sensitivity of HEV NAT assays as part of larger preclinical studies to demonstrate that they are sensitive, specific and reliable for determining the risk of transfusion transmitted HEV. A panel consisting of blood samples from patients with typical symptoms of non-A, non-B, non-C hepatitis (jaundice, fatigue, anorexia, etc.) and elevated ALT that have been virologically confirmed will need to be collected to further characterize such assays. The randomized and coded positive and negative samples could then be sent to laboratories as a validation panel prior to initiation of clinical studies. Further studies with samples from other HEV genotypes associated with human infection will be required for additional characterization of candidate HEV NAT assays.

2. Perform large-scale studies of blood donor samples using well-characterized NAT assays to determine the prevalence of viremic (NAT-positive) blood donors.

Large-scale surveys of U.S. blood donors using validated NAT assays are feasible as a short-term study to determine the prevalence of HEV RNA-positive donors as an estimate of the magnitude of the risk due to HEV exposure and the threat to the safety of the blood supply. A pilot NAT assay with high throughput from a commercial source could be characterized and used for this survey. While large-scale studies also should be done to determine the seroprevalence of anti-HEV in blood donors, they would likely overestimate the risk due to lack of correlation with infectivity.

3. Characterize serologic assays using a panel of virologically confirmed clinical samples.

There are no FDA-approved diagnostic tests for HEV. In-house assays are generally used in the U.S., but there are several commercial assays available in other countries. All of these exhibit a great deal of variability in sensitivity and specificity. This has hindered the collection of consistent and reliable public health data.

There is currently no pedigreed anti-HEV reference panel that could be used to develop highly sensitive and specific serological assays both for anti-HEV IgG and anti-HEV IgM. The current Interim WHO Reference Reagent for hepatitis E serum IgG, established in 2002 from a U.S. citizen infected in India, may be used for evaluating the sensitivity of anti-HEV IgG assays but would not be useful for assessing assay specificity.

There is an international collaborative effort underway to establish a pedigreed panel of HEV sera from patients with HEV infections that have been confirmed by PCR. The samples will be collected from several countries including Europe, the U.S., China, India and Bangladesh, and contain all four genotypes. Samples from both acute and chronic HEV infection with genotype 3 will be included. The panel will then be used to validate existing anti-HEV IgG assays.

The members of the pedigreed anti-HEV panel can be run on various assays to estimate the assays' sensitivities to validate antibody assays. Using the qualified assays, several different types of populations, e.g., blood donors, individuals with non-A, non-B, non-C hepatitis, swine veterinarians and farmers, solid organ donor recipients, etc., can be tested. Availability of this anti-HEV reference panel will allow for properly validated, reliable serological assays that can be used to assist in accurate clinical diagnosis of HEV, in determination of true HEV seroprevalence in the general population and other sub-populations including blood donors, in determination of HEV incidence, and in studies to assess the risk of transfusion transmitted HEV.

4. Perform studies on donor-recipient linked sample repositories using validated NAT assays and serologic assays to assess transfusion transmission of HEV infection.

Based on an assessment of the findings of a NAT survey in blood donors, donor-recipient linked studies can be initiated to indicate whether or not HEV infections are being transmitted.

In addition to NAT testing to identify NAT-positive donors, detection of anti-HEV in transfusion recipients would also be needed to indicate the transmission of HEV infection from the donor. To ensure that transmission of HEV did occur through transfusion, a pre-transfusion sample from the recipient, when available, should be tested for HEV antibody and by HEV NAT, and found to be negative.

Testing of linked donor-recipient samples can be more easily and quickly performed using existing repository samples. The Retrovirus Epidemiology Donor Study (REDS) Allogeneic Donor and Recipient (RADAR) repository is one potential source of linked samples.[78] It contains pre- and post-transfusion specimens collected from 2000-2003 by seven blood centers and eight selected hospitals throughout the U.S. Another possible source of donor-recipient linked samples for a study is the Transfusion-Related Infections Prospective Study (TRIPS) repository. The data from studies using these two repositories could provide important information in determination of the exposure risk of HEV from blood donors and actual transmission to recipients.

Other repository based or prospective studies could be considered.

Summary:

The risk of HEV infection from blood transfusion in the U.S. is unknown and would be difficult to determine due to the large proportion of asymptomatic, undiagnosed HEV infections, the lack of FDA-approved NAT and serologic assays, and unknown seroprevalence and incidence in the general population and in blood donors. HEV poses an increased risk of morbidity and mortality in immunocompromised individuals, pregnant women, and those with pre-existing liver disease, though virulence may vary with the virus genotype. Chronic disease, particularly in solid organ transplant recipients, also makes HEV an important agent of concern because treatment is still evolving and vaccines are not yet available in the U.S. Additional information on basic HEV virology and epidemiology along with development of pedigreed panels and standards to allow for validated NAT assays and serological assays for HEV are required. Studies in blood donors using validated NAT assays are feasible in the short term to evaluate the potential exposure risk to transfusion recipients. Blood donor-transfusion recipient studies using validated serological assays will then be critical to assess whether transfusion transmitted HEV in the U.S. can be confirmed in available repository samples or in prospective studies.

FDA proposes that an assessment of the transfusion risk from HEV could be approached in stages:

1. Characterize NAT assays using the 1st WHO International Standard for HEV RNA and a panel of virologically confirmed clinical samples.

2. Perform large-scale studies of blood donor samples using validated NAT assays to determine the prevalence of viremic (NAT-positive) blood donors.

3. Characterize serologic assays using a panel of virologically confirmed clinical samples.

4. Perform studies on donor-recipient linked sample repositories using well-characterized NAT assays and serologic assays to assess transfusion transmission of HEV infection.

FDA would work to promote the collaborations necessary to achieve these objectives.

Questions for the Committee:

1. Do the available scientific data indicate a need to determine the risk of HEV transmission by transfusions in the U.S.?

2. If so, please comment on FDA's proposed approach to a) assay characterization for NAT and serologic assays; and b) candidate study designs to establish HEV risk from transfusion.

APPENDIX 1: Reported Cases of Transfusion Transmission of HEV Infection

- The first case of transfusion transmitted HEV was reported from Hokkaido, Japan in 2004 in a Japanese male patient who developed acute hepatitis after receiving a transfusion of blood from 23 voluntary donors during open heart surgery. One asymptomatic donor who had no history of travel abroad and a normal alanine aminotransferase (ALT) level at the time of donation was found to be HEV positive. The amplification products of two regions of the HEV from both the patient and donor were sequenced and showed complete identity for genotype 4.[1]

- A Japanese patient received 44 transfusions of red blood cells (RBC) and 40 of platelets from a total of 84 donors during chemotherapy against T-cell lymphoma. He was persistently infected with HEV for approximately 6 months by transfusion of a RBC product. The full-length HEV genome from the donor and recipient were identical and determined to be genotype 3.[2]

- Blood from an asymptomatic Japanese blood donor was found to be positive for HEV RNA. The patient who was transfused with the donation had non-Hodgkin's lymphoma and had undergone autologous peripheral blood stem cell transplantation and received large dosages of chemotherapy. He developed acute hepatitis E. HEV RNA detected in the donor and in the recipient was identical. The donor was determined to be infected with HEV by consumption of grilled meats including pig liver and intestines. HEV RNA was detected in a blood sample from the causative donor's father who developed acute hepatitis and died of fulminant hepatitis. There was a nine nucleotide difference in sequence between the RNA in the donor and his father. This is the first case that identified the HEV infection of the donor as zoonotic foodborne.[3]

- A hemodialysis patient in Japan contracted HEV infection after transfusion with two units of infected blood in 1979. The HEV isolates from both the patient and the donor showed complete identity in the capsid region of the genome that was sequenced and determined to be genotype 3.[4]

- A case of transfusion transmitted HEV in the United Kingdom was from an asymptomatic donor with no travel history or exposure to pigs or the meat industry to one of two recipients. The recipient of the platelets in 3-4 mL of donor plasma was not infected but the recipient of the RBCs in 30 mL of donor plasma, a cancer patient on chemotherapy, was. The donor had flu-like symptoms followed by jaundice but the components had already been transfused. Sequence analysis of the donor and recipient strains were identical and determined to be genotype 3 HEV.[5]

- A 7-year-old boy in France was on chemotherapy for kidney cancer and received 22 transfusions of concentrated erythrocytes or platelets and was diagnosed with HEV infection. Serum samples from 12 units of blood products were tested. One sample from an asymptomatic donor with no history of recent travel abroad was positive for HEV RNA. HEV nucleotide sequences from the donor and recipient were identical and determined to be genotype 3f.[6]

- Potential transfusion transmission of HEV was reported in three patients from four asymptomatic donors in a study from Saudi Arabia. HEV infection developed post-transfusion in 3 of 22 recipients who received blood from 4 asymptomatic donors who were positive for HEV RNA. Three of four donors were also anti-HEV IgM positive. No sequence analysis was performed on the HEV RNA found in the donors and recipients to definitively confirm HEV transfusion transmission. None of the patients who did not receive a transfusion developed infection.[7]

APPENDIX 2: Studies of the Incidence of HEV Infection and Seroprevalence in the U.S.

- The incidence of HEV infection in the general U.S. population has been estimated in one study to be 7 infections per 1000 susceptible persons per year (0.7%). This may be due to the high number of subclinical infections, presence of protective immunity in the host, lack of testing for HEV infection for cases of acute infection or decreased virulence of genotype 3.[65]

- Characteristics of incident HEV cases in the U.S. have not been reported. However, very recently, to enable their description, the Centers for Disease Control and Prevention (CDC) has just completed a study to characterize these occurrences. Individuals who were seronegative for acute hepatitis A and B, whose clinical specimens were referred to the CDC from June 2005 through March 2012 for testing for infection with HEV, were included. Anti-HEV IgM and IgG were tested for in sera, and HEV RNA was tested in sera and stool, and nucleotide sequencing and genotyping were performed. Among 154 persons included, 26 (17%) were identified as cases of hepatitis E of whom 15 cases had not traveled abroad (autochthonous cases) and 11 had recently traveled abroad. Non-travelers compared to travelers were older (median age, 61 vs. 32 years), more likely to be without jaundice (53% vs. 8%) and comprised of more non-South Asians (93% vs. 27%) and more solid organ transplant recipients (47% vs. none). Fulminant hepatic failure was reported in two cases from solid organ transplant recipients. HEV genotype 3 was characterized from the non-travelers (n = 8) and genotype 1 or 4 from the travelers (n = 4). HEV may enter into the differential diagnosis of hepatitis regardless of travel history.[68]

- Another study by the CDC to assess HEV in the U.S. is ongoing. All anti-HEV positive clinical specimens from 39 diagnostic laboratories in 21 states referred to the CDC from October 2009 through October 2011 for confirmatory testing for HEV, were included. Of the 669 cases analyzed, 265 (40%) were anti-HEV IgM positive. The median age of the patients was 43 years (range of 4 to 84 years) with 57% ranging from 21-50 years and 36% greater than 51 years of age. Of the patients, 152 (57%) were male. Further characterization including travel history, mode of transmission and genotype determination has not yet been completed.[69]

- The NHANES III study with testing of over 18,000 samples from 1988-1994 determined HEV seroprevalence to be approximately 21%. This number dropped to 16.8% upon re-testing of a subset of the samples using another anti-HEV IgG test. The CDC tested almost 8000 NHANES IV samples from 2009-2010 and found a seroprevalence of only 6.8%. There is no clear explanation for this decline but variability in sensitivity and specificity of serological and NAT assays, sample dilutions used in the assays or population differences may be contributing factors making any direct comparisons difficult to interpret.[66]

- Several studies to determine the prevalence of anti-HEV in special populations including blood donors have been reported. In the first seroprevalence study for HEV in the U.S., 300 blood donors from Baltimore, MD, 300 from New York

City, NY and 211 from Sacramento, CA were tested for anti-HEV antibodies. Of the samples tested, 21.3%, 31.0% and 13.7% respectively were found to be positive for anti-HEV.[8]

- In another study, 468 veterinarians who work with swine (389 residents of the U.S.) and 400 normal U.S. blood donors were tested for anti-HEV IgG. Anti-HEV prevalence in swine veterinarians from eight U.S. states (Minnesota, Indiana, Nebraska, Iowa, Illinois, Missouri, North Carolina and Alabama) from which normal blood samples were available was determined to be 26% positive when assayed with a human HEV antigen and 23% with a swine HEV antigen. Only 18% of the blood donors from the same eight states were positive with the human HEV antigen and 17% with the swine antigen. The swine veterinarians from this region were 1.51 times more likely when tested with a swine HEV antigen and 1.46 times more likely when tested with a human antigen to be anti-HEV positive than normal blood donors.[9]

- A study ongoing at the National Institutes of Health has found a comparable seroprevalence (22% IgG antibody positive/0.3% IgM antibody positive) after testing of ca. 1000 blood donors. Testing of the anti-HEV antibody positive samples by NAT did not result in detection of HEV RNA. Prevalence of anti-HEV in pre-transfusion samples from 75 recipients was 22.7%. No seroconversion or HEV RNA has been detected after testing of these recipient samples.[10]

- In a study done in 1983 and 2003 in Denmark the prevalence of anti-HEV in farmers and blood donors showed a seroprevalence rate of 50.4% among farmers and 32.9% among blood donors in 1983, and 20.6% among blood donors in 2003. (Farmers were not studied in 2003.)[70]

APPENDIX 3: Studies to Assess the Performance Characteristics of NAT Assays and Serologic Assays

- The performance of assays for the detection of HEV RNA (NAT assays) varies significantly. In a study published last year, a panel of 22 HEV-positive plasma samples (genotypes 3a, 3b, 3f and 4c) obtained from blood donors was tested by 20 labs from 10 countries. All NAT assays used, except one, were developed in-house. There was a 100-1,000 fold difference in sensitivity between the majority of the assays regardless of viral strain tested. Specificity was determined to be high as HEV RNA was not detected in negative samples with the exception of one equivocal result on a replicate sample. The broad variability in assay sensitivity between different laboratories illustrated the need for a well-characterized reference standard for use in standardizing NAT assays to detect and quantify HEV RNA. This study was instrumental in establishing a WHO standard for HEV RNA for NAT assays.[73]

- Results from a collaborative study to evaluate candidate standards for HEV RNA for use in NAT-based assays were published in October 2011. Both genotype 3a and 3b HEV strains obtained from blood donors were considered for the standard. Twenty-four laboratories from 10 countries participated in the study. Data returned from 23 laboratories were analyzed at the Paul-Ehrlich-Institute. Although both candidate standards were detected by all assays, the genotype 3a strain was established as the 1st International Standard for HEV RNA. This standard was estimated to have a potency of 5.39 $\log_{10}$ units/mL and assigned a unitage of 250,000 IU/mL. This assigned unitage is the same as that which was assigned to the Japanese National Standard.[71]

- The CDC recently conducted a study to compare the performance of six serological assays specific to IgM antibodies against HEV (anti-HEV IgM). Four were commercially available and two were in-house assays. Fifty serum samples from acutely jaundiced patients with non-A, B or C hepatitis that were positive for HEV RNA and sequenced for genotype were used as a diagnostic sensitivity panel for the study. Clinical sensitivities of the assays ranged from 72-98% and specificities from 78.5-95.6% for these immunoassays.[74]

- Another recently published study compared the performance of two anti-HEV IgG assays that are commercially available in Europe using known positive sera. One assay was significantly more sensitive in detection of HEV antibodies at 98% compared to 56% for the other assay. The lower limit of detection for HEV IgG was also quite different. One kit detected 0.25 units/mL while the other detected 2.5 units/mL. This was determined by comparison with a 2002 WHO Interim Reference Reagent for hepatitis E serum IgG.[75]

- An earlier study from 1998 reported the performance of 12 assays for detection of antibodies to HEV in a panel of 164 randomized and coded sera. The overall sensitivity of all of the assays varied from 17-100% but the sensitivity of six of the assays was greater than 90%.[76]

- Another study compared three anti-HEV IgM assays commercially available in Europe and reported similar sensitivities of 90, 88 and 82% and nearly identical specificities of 100, 99.5 and 100% in the detection of genotype 3 HEV infections. The authors concluded that this performance was adequate for diagnosis of infection caused by genotype 3 HEV.[77]

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