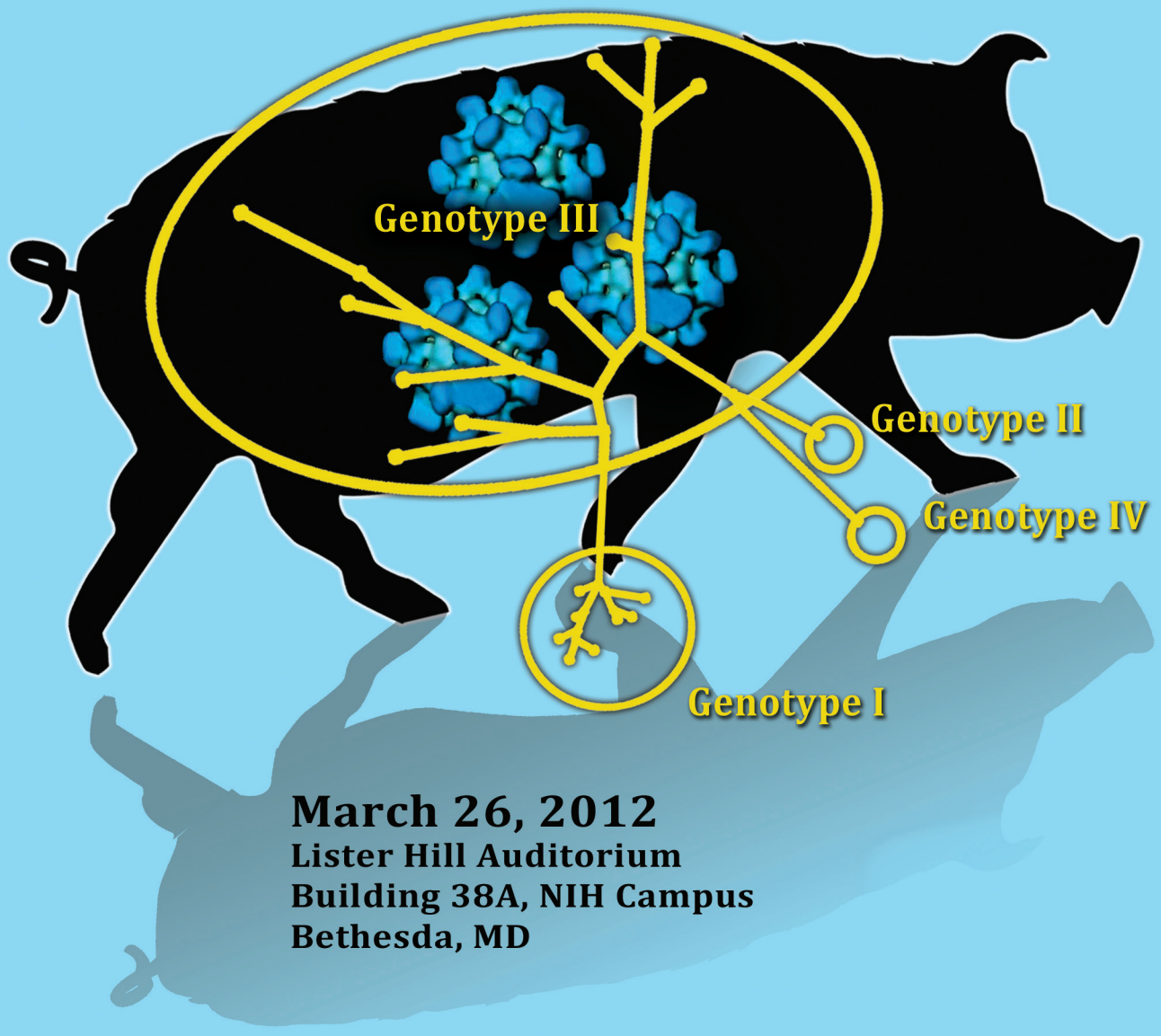
Hepatitis E in the United States

An NIH Research Workshop



March 26, 2012
Lister Hill Auditorium
Building 38A, NIH Campus
Bethesda, MD

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

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NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

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NIAID, NIH

Averell Sherker, M.D., F.R.C.P.(C)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NIH

Jay Hoofnagle, M.D.
NIDDK, NIH

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March 26, 2012

Lister Hill Auditorium
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7:30 a.m. Registration

8:30 a.m. Welcome and Aims
Jay H. Hoofnagle, M.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH)

SESSION 1 THE HEPATITIS E VIRUS (HEV)

Moderators
Rajen Koshy, Ph.D., National Institute of Allergy and Infectious Diseases (NIAID)
Robert Purcell, M.D., NIAID

8:40 a.m. Molecular Virology of HEV
Suzanne Emerson, Ph.D., NIAID, NIH

9:00 a.m. Diagnostic Assays for HEV
Harry Dalton, D. Phil. (Oxon), F.R.C.P., Consultant Gastroenterologist and Honorable Senior Lecturer, European Centre for Environment and Human Health, Peninsula College of Medicine and Dentistry (United Kingdom)

9:20 a.m. Hepatitis E as a Zoonotic Disease in the United States
Xiang-Jin Meng, M.D., Ph.D., Virginia Polytechnic Institute and State University

9:40 a.m. Discussion
Moderators and Speakers

10:10 a.m. Break

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SESSION 2 — EPIDEMIOLOGY OF HEPATITIS E

Moderators
Jay Hoofnagle, M.D., NIDDK
Harry Dalton, D. Phil. (Oxon), F.R.C.P., Consultant Gastroenterologist and Honorable Senior Lecturer, European Centre for Environment and Human Health, Peninsula College of Medicine and Dentistry (United Kingdom)

10:30 a.m. — Hepatitis E, Pigs, and Pregnancy: Recognizing the Global Threat of an Emerging Pathogen
Alain Labrique, Ph.D., M.H.S., M.S., M.A.C.E., The Johns Hopkins University Bloomberg School of Public Health

10:50 a.m. — The Epidemiology of Hepatitis E in Europe
Philippe Colson, M.D., Centre Hospitalo-Universitaire Timone (France)

11:10 a.m. — The Epidemiology of Hepatitis E in the United States
Kenrad Nelson, M.D., The Johns Hopkins University

11:30 a.m. — Hepatitis E in the United States
Scott Holmberg, M.D., M.P.H., Centers for Disease Control and Prevention (CDC)

11:50 a.m. — Discussion
Moderators and Speakers

12:20 p.m. — Lunch and Posters

SESSION 3 — SPECIAL POPULATIONS

Moderators
Kenrad Nelson, M.D., The Johns Hopkins University
Averell Sherker, M.D., F.R.C.P.(C), NIDDK

1:30 p.m. — Hepatitis E in the United States: Clinical Course and Complications
Jay H. Hoofnagle, M.D., NIDDK, NIH

1:50 p.m. — Hepatitis E as a Cause of Acute Liver Failure in the United States
Robert Fontana, M.D., University of Michigan Medical School

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2:10 p.m.	Hepatitis E After Organ Transplantation *Norah Terrault, M.D., University of California, San Francisco*
2:30 p.m.	Hepatitis E Infection in HIV-infected Patients *Caryn Morse, M.D., M.P.H., Warren G. Magnuson Clinical Center (CC), NIH*
2:50 p.m.	Discussion *Moderators and Speakers*
3:20 p.m.	Break
3:40 p.m.	An Assessment of HEV Prevalence and Risk in U.S. Blood Donors and Recipients *Chenyu Xu, Ph.D., Department of Transfusion Medicine, NIH*
4:00 p.m.	HEV and Food Safety Issues *Julie Ann Kase, Ph.D., FDA*
4:20 p.m.	HEV Vaccine Strategies: Utility and Prospect *James Wai Kuo Shih, Ph.D., Xiamen University (China)*
4:40 p.m.	Treatment of Hepatitis E Virus Infection *Nassim Kamar, M.D., Ph.D., Centre Hospitalier Universitaire Rangueil (France)*
5:00 p.m.	Discussion of Future Needs *Moderators and Speakers*
5:30 p.m.	Adjournment

Speaker Abstracts

Molecular Virology of HEV

Suzanne Emerson, Ph.D.

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Hepatitis E virus (HEV, genus *Hepevirus,* family *Hepeviridae*) is a non-enveloped, icosahedral particle that encloses a positive-sense RNA genome of 7.2kb. The capsid gene (ORF2) encodes a protein of 660 amino acids that includes a putative N-terminal signal sequence and 3 internal glycosylation motifs. The size and composition of ORF2 protein in virions is still unknown.

The 4 genotypes (> 80% sequence homology within a genotype) of HEV that infect humans comprise a single serotype, and neutralizing antibodies to genotype 1 capsid are cross-protective. Crystal structure determination of recombinant virus-like particles revealed homodimers with 3 domains: S (shell), M (middle), and P (protruding). ORF2 protein homodimerization is required to generate the neutralization epitopes, which are conformational and located on the P domain. Virions in the blood or produced in cell culture are associated with lipids and with the small viral ORF3 protein of 113-114 amino acids. Virions in the feces lack ORF3 protein, which may be removed during excretion through the bile duct.

The HEV 7.2kb genome contains three open reading frames and has a methylated guanosine cap at the 5' end, a short non-translated region at each terminus, and a polyadenosine tract at the 3' end. The largest reading frame, ORF1, serves as the messenger RNA for the non-structural proteins required for RNA replication and for subgenomic RNA synthesis and is most likely translated immediately following RNA uncoating in the cytoplasm. ORF2 capsid protein and ORF3 protein are both synthesized from the same bicistronic, subgenomic RNA of 2.2kb.

The viral replicative cycle has not been thoroughly studied and is mostly predicted by analogy with those of better known viruses. The cellular receptor(s) is unknown. The virus replicates in the cytoplasm and is released from cells by an unknown mechanism which involves ORF3 protein. HEV is believed to be non-cytopathic.

Cell culture systems and infectious cDNA clones have been developed only recently, and only for genotypes 3 and 4. A genotype 3 and a genotype 4 strain were adapted by Okamoto and colleagues to growth in A549 human lung cells and PLC/PRF/5 human hepatoma cells. A second genotype 3 strain was adapted by Emerson and colleagues to growth in HepG2/C3A human hepatoma cells. This virus will also infect and replicate in kidney cells from swine, the major zoonotic host for genotype 3 viruses. This cell culture-adapted strain is a naturally-occurring recombinant virus that has acquired a human sequence which promotes growth *in vitro*.

Infection normally occurs via ingestion of contaminated water or under-cooked pork or game meat. The liver is the main target organ, but it is not clear how the infecting virus reaches the liver or what constitutes the basis for the tropism. Viremia is usual, and viruses are excreted into the bile duct and shed in the feces. HEV in swine has been isolated from other tissues, in addition to liver, suggesting that other sites of replication may exist. Interestingly, the virus has also been isolated from the cerebrospinal fluid of a chronically infected patient. The importance of extra-hepatic replication sites remains to be determined.

The number of newly discovered HEV strains and HEV-like viruses is increasing rapidly but insufficient characterization has occurred to provide a comprehensive picture of the diversity and scope of the family. Clearly though, the *Hepeviridae* viruses are much more complicated and widespread than imagined even a few years ago.

Molecular Virology of HEV

CONTINUED

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Diagnostic Assays for HEV

Harry Dalton, D.Phil. (Oxon), F.R.C.P.

Consultant Gastroenterologist and Honorable Senior Lecturer, European Centre for Environment and Human Health, Peninsula College of Medicine and Dentistry, Truro, United Kingdom

Locally acquired hepatitis E virus (HEV) infection has almost certainly been in our midst in developed countries (including the United States) for decades (1,2). One of the reasons we failed to recognise this fact until recently is that the diagnostic tests in general, and serology in particular, were of very poor quality, with poor sensitivity. This has almost certainly led to a significant underestimate of the HEV seroprevalence in developed countries in many studies published to date, and coloured our view that "HEV is not much of a problem for us."

Molecular Assays

Polymerase chain reaction (PCR) assays on serum and/or stool are important diagnostic tools. However, in the context of acute HEV infection, the period of viraemia maybe brief, and so a negative PCR reaction for HEV RNA does not exclude the diagnosis. In chronic HEV infection, HEV RNA testing is essential for making the diagnosis, as serology in the immunosuppressed individual is unreliable. PCR for HEV RNA is also essential for monitoring therapy in patients with chronic HEV infection. However, the molecular assays for HEV diagnosis and monitoring have not been standardised between laboratories, nor between countries, and the performance of these assays varies considerably from laboratory to laboratory.

Serological Assays

Commercially available and "in-house" serological assays vary widely in sensitivity and specificity, and there is poor inter-assay concordance. There is no "gold standard."

Using a large serum bank of acute and convalescent sera from PCR proven cases of locally acquired HEV3 in the United Kingdom, we performed a detailed comparison of two commercially available HEV serological assays. The first assay used GL antigen as a target. This is based on a mixture of ORF2 peptides from HEV1 (Burma) and HEV2 (Mexico) and an ORF3 recombinant peptide. The second assay (PE2) is a recombinant dimeric peptide derived from HEV1 (China) and is similar to the antigen used in the HEV239 vaccine. The sensitivity of the GL assay was 56% and the PE2 assay 98%. The reasons for these differences related in part to the higher sensitivity of the GL assay being 10 times higher (2.5 WHO units vs. 0.25 WHO units). However, there were also qualitative differences between the assays. Applied to blood donors in Cornwall, United Kingdom, GL assay gave a seroprevalence of 3.6%, compared to PE2 assay, which gave a seroprevalence of 16.2% (3).

Relevance to the Existing Literature

There have been many studies of HEV seroprevalence in developed countries over the past 15 years. The results are very variable, with some studies describing a seroprevalence of < 1% and others (notably the National Health and Nutrition Examination Survey (NHANES) study in the United States) describing a seroprevalence of > 20%. The reasons for these widely divergent results are uncertain. There is some evidence that suggests that HEV seroprevalence varies between and within developed countries. However, many of the studies reported in the literature used insensitive assays that employed the GL antigen (or a variation thereof) as the target antigen. Such studies need to be interpreted with caution. For example, a study from Toulouse, France, showed that the seroprevalence in blood donors was 16% using a GL-based assay. A re-examination using the PE2-based assay gave a much higher result, indicating the HEV is hyper-endemic in the Toulouse area (4). The latter result would fit with the observed incidence of HEV infection (using PCR-based techniques) in the Toulouse transplant population of 3.2% (5).

The Way Forward

We need to establish, as a matter of priority, an

Diagnostic Assays for HEV

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unimpeachable bank of acute and convalescent sera from proven cases of HEV3 infection. Such a serum panel could be used as an international resource to develop sensitive and specific assays. This serum bank should:

- Be from cases shown to be HEV RNA positive.
- Contain cases identified using different screening methodologies from diverse geographical locations.
- Contain samples of large volume.

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(1) Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol* 2008 Mar; 48(3): 494-503.

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Hepatitis E as a Zoonotic Disease in the United States

X.J. Meng, M.D., Ph.D.

Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

With the genetic identification of various animal strains of hepatitis E virus (HEV) from pig, chicken, rabbit, deer, mongoose, and fish, the host range and genetic diversity of HEV have been significantly expanded. Thus far, at least four recognized and two further putative genotypes of HEV have been identified in mammalian species (Table 1): genotypes 1 and 2 are restricted in humans, whereas genotypes 3 and 4 are zoonotic. Two putative new genotypes of mammalian HEV have recently been identified from rats in Germany and the United States, and from wild boars in Japan. In addition, novel strains of HEV have also been genetically identified from non-mammalian species including chickens and fish that may represent two separate new genera within the family *Hepeviridae*. In the United States, animal strains of HEV have been genetically identified from pigs, chickens, rabbits, rats, and fish.

With the exception of swine hepatitis E virus (swine HEV), which is known to be zoonotic and transmissible to man, the zoonotic potential of other animal strains of HEV in the United States is unclear.

Swine HEV was first identified and characterized from pigs in the United States (1). Swine HEV infection in pigs is widespread in the United States, with more than 80% of pigs infected in some herds. Thus far, all HEV strains identified from pigs in the United States belong to genotype 3, even though genotypes 3 and 4 as well as a putative new genotype have been identified from pigs in other countries. Approximately 11% of the commercial pig livers from local grocery stores in the United States contain infectious HEV. Although not yet reported in the United States, cluster and sporadic cases of acute hepatitis E directly linking to the consumption of undercooked pig livers have been reported in other countries. In the United States, the prevalence of HEV antibodies in pig handlers (veterinarians and farmers) is significantly higher than that in age- and geography-matched normal blood donors, and pig handlers are at increased risk of zoonotic HEV infection.

Table 1. Proposed Classification, Host Range, and Cross-species Infection of HEV

HEV Strains	Natural Hosts	Experimental Hosts
Genus *Hepevirus*		
Genotype 1	human	monkey, rat, lamb
Genotype 2	human	monkey
Genotype 3	human, pig, deer, mongoose, rabbit	monkey, pig
Genotype 4	human, pig	monkey, pig
Putative genotype 5	rats	
Putative genotype 6	wild boar	
Putative Genus *Avihepevirus*		
Genotype 1	chicken (Australia)	turkey, chicken
Genotype 2	chicken (USA)	
Genotype 3	chicken (Europe and China)	
Putative Genus *Piscihepevirus*		
Cutthroat trout virus	fish	

Hepatitis E as a Zoonotic Disease in the United States

CONTINUED

Avian hepatitis E virus (avian HEV) was identified from chickens with Hepatitis-Splenomegaly syndrome in the United States (2), and is genetically and antigenically related to the Big Liver and Spleen disease virus (BLSV) from chickens in Australia. Avian HEV shares approximately 50% nucleotide sequence identity with mammalian HEV across the entire genome. At least three genetically distinct genotypes of avian HEV have been identified from chickens worldwide: genotype 1 (Australia), genotype 2 (USA), and genotype 3 (Europe and China). Like swine HEV, avian HEV also has the ability to cross species barriers and infect turkeys under experimental conditions, although avian HEV failed to infect rhesus monkeys. Therefore, it appears that avian HEV has a more limited host range and is likely not transmissible to humans.

Novel strains of HEV were identified genetically from farmed rabbits first in China and subsequently in the United States (3). The prevalence of HEV infection in rabbits in the United States is high: HEV RNA was detected in 16% of serum and 15% of fecal samples, and IgG anti-HEV in 36% of sera from two rabbit farms in Virginia. Sequence and phylogenetic analyses revealed that the rabbit HEV from farmed rabbits in the United States and China is a distant member of the genotype 3. Cross-species infection of pigs by the rabbit HEV has recently been demonstrated (Cossaboom et al., unpublished data). Therefore, as a genotype 3 HEV strain, the rabbit HEV in the United States may be zoonotic and thus poses a potential zoonotic concern.

Another novel strain of HEV was recently identified from rats in the United States (Los Angeles, CA) (4) and shown to be genetically similar to the HEV strain identified from rats in Germany. The rat HEV strains identified thus far appear to belong to a putative new genotype that is distinct from the four recognized genotypes of mammalian HEV. Attempts to experimentally infect rhesus macaques and pigs with the U.S. strain of rat HEV were unsuccessful, suggesting that the rat HEV identified thus far from USA and Germany has a limited host range and is likely not zoonotic. However, the extent of HEV infection in wild rats by other known or unknown genotypes of HEV is currently unclear and warrants further investigation.

A novel virus, designated cutthroat trout virus (CTV), was isolated from spawning adult trout in the United States in the Chinook salmon embryo (CHSE-214) cell line (5). The genomic organization of CTV is similar to that of HEV, and shared 13-27% amino acid sequence identity with known HEV strains. It appears that CTV in fish may belong to another new genus within the family *Hepeviridae*.

In summary, hepatitis E is a recognized zoonotic disease, and pigs and likely other animal species are reservoirs for HEV. In the United States, the genotype 3 HEV from animals (pigs and rabbits) is the main concern for zoonotic infection. These animal strains of HEV also raise concerns over food and environmental safety.

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(2) Haqshenas G, Shivaprasad HL, Woolcock PR, et al. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *J Gen Virol* 2011; 82: 2449-62.

(3) Cossaboom CM, Cordoba L, Dryman BA, et al. Hepatitis E virus in rabbits, Virginia, USA. *Emerg Infect Dis* 2011; 17(11): 2047-9.

(4) Purcell RH, Engle RE, Rood MP, et al. Hepatitis E virus in rats, Los Angeles, California, USA. *Emerg Infect Dis* 2011; 17(12): 2216-22.

(5) Batts W, Yun S, Hedrick R, et al. A novel member of the family *Hepeviridae* from cutthroat trout (Oncorhynchus clarkii). *Virus Res* 2011; 158: 116-23.

Hepatitis E, Pigs and Pregnancy: Recognizing the Global Threat of an Emerging Pathogen

Alain B. Labrique, Ph.D., M.H.S., M.S., M.A.C.E.[1], Sabra Klein[1], Brittany Kmush[1], Hasmot Ali[2], Ronald E. Engle[3], Kerry Schulze[1], Keith P. West[1], Jr., Robert H. Purcell, and Kenrad E. Nelson[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]The JiVitA Maternal and Child Health Research Project, Gaibandha, Bangladesh; [3]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, USA

Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis globally and results in severe morbidity and mortality in pregnant women. Over the past decade, paradigm shifts have changed our understanding of the complex epidemiology of this pathogen. Depending on genotype, hepatitis E viruses are responsible for substantial human infections through enteric, fecal-oral, or foodborne routes. Across the continent of Asia, dominant genotypes result in primarily large human outbreaks of disease (gt 1) or elevated caseloads of sporadic illness (gts 3 / 4), and sometimes even both in populations where human sanitation levels are low, and high-risk animal husbandry practices exist. HEV infections and their severe consequences in pregnancy remain a source of major public health concern. In addition to a review of the epidemiology and burden of HEV disease in Asia, we will share some novel data that may shed light on the elevated risk of hepatitis E-associated complications in pregnancy.

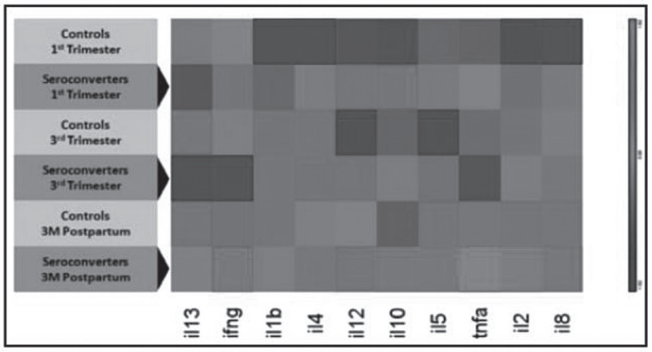

Figure 1: Heat map generated using Partek to illustrate the mean concentrations of cytokines (normalized for each group to 0, with standard deviation normalized to 1). All seroconversions occurred between the 3rd trimester and the 3M Postpartum visit. Here, darker red cells reflect high upregulation / expression, and blue reflects relative downregulation / absence.

There is a paucity of longitudinal data examining the incidence and disease rate of HEV in cohorts of pregnancy in endemic areas. We studied serial sera collected within two prospective cohorts totaling 110,473 incident pregnancies enrolled in large randomized trials in rural northwestern Bangladesh, between 2001–2007 (cohort A) and 2007–2010 (cohort B). An NIH research immunoassay was used to identify anti-HEV IgG status in early pregnancy, late pregnancy, and 3-month postpartum venous blood specimens, drawn on a subsample of the larger cohorts. Of the 1,127 specimens available for testing in cohort A, 72 were anti-HEV seropositive at baseline, indicating a seroprevalence of ~6.4%. During this period, 63 women were identified as potential seroconverters, suggesting an incidence rate of ~56 infections per 1,000 person-years. In the more recent cohort B, 1,100 were available for testing, revealing a ~6.1% seroprevalence in anti-HEV IgG at early pregnancy. Within this cohort, 40 women were identified as putative seroconverters, an incidence rate of 46 infections per 1,000 person-years. Between the 2001 to 2006 cohort and the 2008 to 2010 cohort, the incidence of intrapartum HEV infections seems to be declining in rural Bangladesh, possibly reflective of improved population sanitation or nutritional status. In these cohorts, sensitive cytokine analysis (10-plex MSD) of infected cases matched by age and parity to uninfected controls has uncovered unexpected patterns of

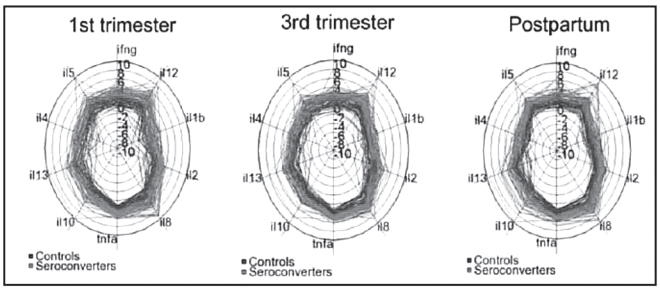

Figure 3. Star plots of cytokine concentrations from all individuals in the study during the 1st and 3rd trimester and 3 months postpartum. Data are grouped, as in Figure 2, as anti-inflammatory (left) and pro-inflammatory (right) cytokines. Controls are plotted in blue, and seroconverters in red.

Hepatitis E, Pigs and Pregnancy: Recognizing the Global Threat of an Emerging Pathogen

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immunologic dysregulation in early pregnancy associated with micronutrient deficiencies (zinc, vitamin D), and risk of late pregnancy infection (see figures, above). Overall upregulation of pro- and anti-inflammatory cytokines was observed in cases when compared to matched seronegative controls. Our data also suggest a failure in this population to mount the dramatic Th1 to Th2 shifts in immunologic character in pregnancy, thought to be needed for optimal fetal development and successful parturition. Women who become infected with HEV in late pregnancy *begin* pregnancy as micronutrient deficient and exhibit characteristics of immune dysregulation. This suggests a possible novel paradigm of explaining intrapartum HEV infections, disease, and perhaps also mortality, which may not be entirely attributable to virus-induced host immune modification or an inappropriate, pregnancy-induced, immunopathologic response.

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The Epidemiology of Hepatitis E in Europe

Philippe Colson, M.D.

Centre Hospitalo-Universitaire Timone, Assistance Publique des Hôpitaux de Marseille, Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique; Aix-Marseille Université, URMITE CNRS UMR 6236 IRD 198, Méditerranée Infection, Facultés de Médecine et de Pharmacie, Marseille, France

Our understanding of the epidemiology of hepatitis E virus (HEV) in Europe has changed significantly over the past decade. Thus, until recently it was thought that HEV infections occurring in Europe were related mainly to travel in the tropics or subtropics; yet recent findings indicate that most cases are autochthonous, occurring in patients who have not traveled abroad. Furthermore, it was shown that there is a major porcine reservoir for HEV that represents a source of viral transmission. The number of diagnosed autochthnous HEV infections in Europe has increased more than 10 fold since 2005, representing several hundred cases each year nationally. In addition the reported prevalence of anti-HEV IgG antibodies has become higher than expected, reaching 20% or higher, with considerable variation between reports. Moreover, recent serological studies suggest that exposure to HEV may still be considerably underestimated. In Southern France, the prevalence of anti-HEV IgG was recently found to be 53% among blood donors, which is 3.1 times higher than previously assessed (17%) in the same population. The prevalence of anti-HEV IgG increased with age and was associated with rural residence, hunting and consumption of game meat. A large body of data indicates that HEV infection is a porcine zoonosis in Europe, pigs and wild boars being commonly infected. Moreover, HEV RNA was detected in 2%–7% of pieces of pig liver that enter the food chain, and in about half of pig liver sausages. Eating game meat has been statistically significantly associated with HEV infection, and HEV transmissions through consumption of inadequately cooked pig liver sausage have been reported. The virus also has been detected in water without evidence of transmission to humans, and foodborne transmission of HEV linked to shellfish consumption has been suspected from epidemiologic analyses of outbreaks. Rare cases of infection through blood transfusion have been reported and linked to asymptomatic mild, acute hepatitis E in the donor. HEV strains that infect pigs and are involved in autochthonous infections in Europe belong to genotype 3 in almost all cases; subtype 3f being the most frequently identified followed by subtypes 3c and 3e. However, infections with HEV genotype 4, the other zoonotic form of HEV that has been increasingly described in China in sporadic hepatitis E and pigs, have been recently documented.

The emergence of autochthonous HEV infections in Europe over the last decade may be merely the result of improved HEV diagnosis tests or an increased awareness of clinicians leading to increased HEV testing, rather than an actual increase in the frequency of infection. The implementation of molecular diagnosis of HEV in clinical virology laboratories probably contributed much to the increased numbers of reports of HEV infections. It is also likely that clinicians are now more likely to test for anti-HEV in patients with liver injury, regardless of whether patients have traveled abroad. This increased suspicion of HEV infection was spurred by the growing awareness that HEV can be acquired in absence of travel abroad and that acute HEV infection can lead to a fatal outcome and may progress to chronic hepatitis and cirrhosis in persons with severe immunodeficiency. The increased reports of acute hepatitis E in the developed world has also led to a renewed interest in research on HEV: publications on hepatitis E in Europe increased 7 times over the last decade and numbers of HEV sequences submitted to GenBank increased 10 fold in 2011 compared to the late 1990s. Regarding data on the time trend of HEV infections, the prevalence of anti-HEV IgG among blood donors in Denmark was reported to decrease from 33% to 21% between 1983 and 2003. Regardless, there remains a discrepancy bettwen the frequency of anti-HEV IgG in the population and the small number of cases of clinically apparent hepatitis E. Thesel findings suggest that the proportion of subclinical infections with HEV must be high. Furthermore, HEV has been retrospectively shown to be a misdiagnosed cause of acute hepatitis and liver injury. Another reason for the above discrepancy may include a low specificity of anti-HEV IgG

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assays.

Overall, the epidemiology of HEV infection in Europe is still not completely understood but its knowledge has improved considerably, and this virus has now its place alongside the other hepatitis viruses as a significant causative agent of acute and chronic hepatitis with a major porcine reservoir that represents a source for foodborne viral transmission.

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The Epidemiology of Hepatitis E in the United States

Kenrad E. Nelson, M.D.[1,2]; Mark Kuniholm, Ph.D.[1]; and Alain Labrique, Ph.D.[1,2]

Departments of [1]Epidemiology and [2]International Health, The Johns Hopkins University School of Public Health, Baltimore, Maryland, USA

Until recently, very few patients with clinical hepatitis from hepatitis E virus (HEV) infection have been recognized in the United States. Among patients with symptomatic HEV, many were in persons with a recent travel history to countries where HEV is endemic. In Western Europe, where serologic testing is more widely available in contrast to the unavailability of anti-HEV serological assays in the United States, an increasing number of both travel-associated and autochthonous cases of HEV have been recognized in the past decade. However, several autochthonous cases have been reported very recently among U.S. residents. Evidence of HEV infection has also been found in some patients diagnosed, sometimes incorrectly, to have drug-induced liver disease. In order to evaluate the HEV seroprevalence among persons living in the United States, we tested the sera for anti-HEV IgG from 18,695 persons enrolled in the 3rd NHANES, a systematic sample of the non-institutionalized civilian population in 1988-1994. The HEV seroprevalence was 21.0% (95% CI 19.0-22.9%).

Among U.S.-born individuals, males, non-Hispanic whites and persons residing in the Midwest and/or in metropolitan areas had the highest seroprevalence. Persons born in Mexico had a higher seroprevalence of 30.9% (95% CI 18.3-22.3), than those born in the United States, with 20.1% (95% CI 18.1-22.0). Seroprevalence increased with age and was higher in males. Having a pet in the home was a risk factor for increased prevalence, OR 1.19 (1.01-1.40), and consuming liver or other organ meats more than once per month was also significantly associated with HEV seropostivity, OR 1.38 (95% CI 1.01-1.88). These data, together with reports of the identification of HEV RNA from pig liver for sale at food stores and higher HEV seroprevalence reported among veterinarians and swine farmers in the United States, suggests that HEV has a zoonotic reservoir. Nevertheless, the specific exposures causing HEV in most U.S. cases has been unclear. However, the potential for exposures to HEV infection includes not only consumption of pig liver or intestine but also contamination of other food by swine feces on the farm and cross contamination at a market or in the kitchen.

HEV infections among residents of the United States appear to be common but have been infrequently diagnosed. Most infections in healthy persons may be asymptomatic; however, the lack of a licensed serologic assay has prevented the diagnosis of many patients with HEV infection.

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Hepatitits E in the United States

Scott D. Holmberg, M.D., M.P.H.

Centers for Disease Control and Prevention, Atlanta, Georgia, USA

In the United States, asymptomatic but apparently widespread infections with hepatitis E virus (HEV) genotype 3 are observed. A previous analysis showed that 21% of 18,695 persons aged 6 years or older in the National Health and Nutrition Examination Survey (NHANES) III in 1988-1994 had serologic evidence of prior HEV infection (1). Because symptomatic hepatitis E is observed so infrequently, there was speculation that the assay used for testing was not sufficiently specific. However, recent retesting of 5,970 samples from NHANES III with a more specific assay (2) showed that a similarly sizable percentage (16.8%) of specimens tested positive for IgG anti-HEV. However, testing of 7,885 NHANES IV samples from 2009-2010 showed that only 6.4% were seropositive. Preliminary analysis of the NHANES IV data is remarkable that, while seroprevalence now appears to be much lower about 20 years later, both the NHANES III and IV serum show: 1) strong increasing seroprevalence with increasing age; 2) substantially increased likelihood of seroprevalence (50% more) among Mexican-born residents; and 3) substantially reduced seroprevalence (40% less) among black residents compared with (non-Hispanic) white residents. From the earlier NHANES III analysis and from the NHANES IV analysis to date, no other strong association indicating the source(s) of this widespread infection has been observed.

HEV genotype 3 has been repeatedly isolated from feral and domestic pigs, but proving this zoonotic source of human infections has been elusive. Studies of persons with occupational exposure to pigs—such as abbatoir workers and farmers—show that they have up to twice the seropositivity than the still considerable seropositivity (21%) in persons without that exposure. However, relatively few persons have such occupational exposure. NHANES participants who infrequently or never ate pork were only slightly less likely to be HEV-seropositive than those who did not, and while those who ate organ meats were more likely to be seropositive than those who did not, organ meats in the United States are usually consumed cooked; thus this exposure would not account for the high HEV seroprevalence. Findings from various studies are similarly weak, confusing, or both, such as no or weak associations with activities associated with fecal-oral, bloodborne, and sexual transmission, such as pet cat or dog ownership; male-male sex; non-injection or injection drug use; number of lifetime sex partners; or source of drinking water (actually, less in well water-users). Similarly, other sources of information have not been especially illuminating.

The U.S. Centers for Disease Control and Prevention (CDC) Viral Hepatitis Laboratory typically receives 12-15 specimens per year—totaling about 180 tests since 1998—for confirmation of initial HEV positive test results at external laboratories, but almost all of these "positive" results could not be replicated at CDC. Of 123 samples from U.S. suspected cases from 2005 to the present, only 27 (22%) were confirmed by CDC's laboratory. Of these cases, the mean age was 42 years (range, 12-68 years); 59% were male; 8 (30%) were Asian; 4 (15%) were Hispanic; 7 (26%) were organ-transplant recipients; 10 (37%) had history of travel to endemic countries (usually in Asia); and of the 17 non-travelers, 6 were gt 3. Two recently investigated case-patients in San Antonio, Texas, illustrate the difficulty in identifying sources of infection (3). One, a 21-year-old Hispanic woman, who had a history of a positive home pregnancy test and heavy alcohol use in the 5 months before illness, developed severe hepatitis and died. The second, a 44-year-old Hispanic woman, also with a history of heavy alcohol use, had a protracted illness with severe hepatitis. These two women, located about 10 km apart, had no common exposures, did not use the same restaurants or

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supermarkets, and 13 of their close contacts who were tested were HEV-negative. Neither had a history of raw/undercooked pork consumption, travel outside the city, or game meat/wild animal exposure. However, the second case had eaten raw oysters 2 months previous.

Given that HEV is probably spread fecal-orally or is foodborne, other factors may need to be examined or other types of studies may need to be undertaken, for example, testing of vegetables and foods potentially contaminated by feral pigs; studies of shedding of virus in identified acutely infected persons (potential for person-to-person spread); greater examination of mollusks and shellfish as potential sources of infection; and better alcohol use and dietary histories for infected persons with symptoms. As of this date, hepatitis "E" stands for "enigma."

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Hepatitis E in the United States: Clinical Course and Complications

Jay H. Hoofnagle, M.D.

Director, Liver Disease Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Bethesda, Maryland, USA

Hepatitis E is caused by a small single-stranded RNA virus (HEV) that belongs to the family *Hepeviridae*. The disease that this infection causes resembles other forms of viral hepatitis. The incubation period ranges from 3 to 8 weeks, and the disease usually presents with a 3 to 7 day prodrome of fatigue, nausea, and right upper quadrant discomfort followed by dark urine and jaundice. The severity of the illness varies widely, but hepatitis E is typically self-limiting and clinical recovery occurs within 2 to 8 weeks.

There are two major epidemiologic and clinical forms of hepatitis E: (1) the classic epidemic form of hepatitis E that is common in the developing world, occurs both sporadically and in large water-borne epidemics, and is caused by genotype 1 or 2 HEV, viruses which largely infect humans and higher primates; and (2) an endemic or autochthonous form of hepatitis E that occurs sporadically in the developed world, usually as a result of food or zoonotic exposure, and is caused by genotype 3 and 4 HEV, which primarily infects swine but can also infect other mammalian species as well as humans. Genotype 1 and 2 HEV is largely a human disease, is highly contagious, typically occurs in young adults, and its clinical features are marked by a high rate of jaundice, cholestatic course, and high fatality rate in pregnant women. In contrast, genotype 3 and 4 HEV is largely a disease of swine, humans being an accidental host, the clinical disease being typically anicteric and mild, most often clinically apparent in older men and becoming chronic in a proportion of immune-suppressed persons.

The clinical, biochemical, and virological course of hepatitis E has been well defined and actually resembles that of other forms of viral hepatitis. Hepatitis E virus (as shown by the presence of HEV RNA) appears in the liver, stool, and blood during the clinical incubation period of disease, rises rapidly in titer, and peaks at about the time of onset of serum enzyme elevations and jaundice. Virus levels decline during the course of illness and disappear in convalescence. Antibody to HEV arises at the time of onset of clinical symptoms, and the initial form is both IgM and IgG anti-HEV. IgM anti-HEV titers peak early and begin to disappear with convalescence while IgG titers rise and persist. The diagnosis of acute hepatitis E is best made by the finding of IgM anti-HEV and is confirmed by identifying HEV RNA in blood or stool or by subsequent rise in IgG anti-HEV and fall in IgM reactivity. At present, the major challenge in diagnosis is the lack of reliable, U.S. Food and Drug Administration (FDA)-approved assays for IgM and IgG anti-HEV and for HEV RNA detection.

At least 20% of the adult U.S. population has IgG antibody to HEV (anti-HEV), yet only a small proportion have a clinical history of acute hepatitis or jaundice, suggesting a high rate of subclinical infections. Thus, the most typical presentation of genotype 3 and 4 HEV infection is likely to be a transient, anicteric, and asymptomatic hepatitis that goes unnoticed or is identified incidentally or as a result of serological screening. Nevertheless, genotype 3 and 4 HEV infection can be clinically manifest and is most common in older men. This propensity suggests an unusual susceptibility to more severe hepatitis with male sex and age. In addition, HEV infection can become chronic, particularly in immune deficient or suppressed individuals (in 30% to 60% of cases). Interestingly, chronic HEV infection can ultimately resolve either in response to immune reconstitution (or reduction in immune suppression) or with antiviral therapy such as with peginterferon or ribavirin or both. Finally, HEV infection can manifest in unusual fashion such as "acute-on-chronic" liver disease in which a patient with mild or even unsuspected chronic liver disease presents with hepatic decompensation at the time of onset of jaundice and symptoms. Hepatitis E can also cause acute liver failure, although usually in the elderly rather than in

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pregnant women. Finally, hepatitis E is associated with uncommon, but occasionally prominent and disabling extra-hepatic manifestations, including maculopapular rash, arthralgias-arthritis, pancreatitis, pure red cell dysplasia, and striking neurological complications such as polyradicular neuropathy, Guillain Barre syndrome, Bell's palsy, ataxia, seizures, and encephalitis.

Thus, hepatitis E is not uncommon in the Western, developed world and deserves to be more commonly considered in patients presenting with acute hepatitis, particularly the elderly. Hepatitis E can be fatal and can evolve into chronic hepatitis and result in cirrhosis and end-stage liver disease. Importantly, hepatitis E is likely to be an easily preventable illness and as well as treatable using antiviral agents that are currently available.

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Hepatitis E as a Cause of Acute Liver Failure in the United States

Robert J. Fontana, M.D.[1]; Ronald E. Engle, M.S.[2]; William M. Lee, M.D.[3]; Holly Battenhouse, M.S.[4]; Robert Purcell, M.D.[2]

[1]University of Michigan Medical School, Ann Arbor, Michigan, USA; [2]National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland, USA; [3]The University of Texas Southwestern Medical Center (UTSW), Dallas, Texas, USA; [4]Medical University of South Carolina (MUSC), Charleston, South Carolina, USA

The U.S. Acute Liver Failure Study Group (ALFSG) has enrolled over 1,800 adult subjects since 1998 into its ongoing registry study of the etiologies and outcomes of ALF patients. A robust database of clinical, demographic, and laboratory features are available as well as serum, urine, and DNA samples from subjects collected during study days 1 to 7 and at 1- and 2-year long-term follow-up visits (1). Approximately, 10 to 15% of patients have been categorized as "indeterminate" ALF with no identifiable etiology. Although some indeterminate patients may have unrecognized acetaminophen overdose or autoimmune hepatitis, the possibility of an occult viral infection remains tenable (1).

Acute sporadic HEV infection in western countries is uncommon and in the absence of a recent foreign travel history is believed to be an autochthonous infection wherein genotype 3 HEV is transmitted through consumption of undercooked pork and/or wild game (2). The Drug Induced Liver Injury Network (DILIN) in collaboration with Dr. Purcell's laboratory at the National Institute of Allergy and Infectious Diseases (NIAID) recently demonstrated that 9 of 318 (3%) consecutively enrolled U.S. patients with suspected DILI were anti-HEV IgM positive, four of these patients had detectable hepatitis E virus (HEV) RNA, and four also demonstrated a rising titer of anti-HEV IgG at 6 months of follow-up consistent with recent acute HEV infection (3). These data suggest that acute sporadic HEV infection may be present in the general U.S. population.

We hypothesized that acute HEV infection may account for some cases of indeterminate and drug-induced ALF in the U.S. ALFSG. In 2004, the ALFSG tested the serum samples of 126 ALF patients for HEV RNA by PCR and for anti-HEV IgM and IgG ELISA using an ELISA assay based upon two recombinant antigens derived from a Burmese isolate of HEV (4). Although 11 patient samples demonstrated some reactivity to one or more HEV peptides, none of them demonstrated any detectable HEV RNA and the pattern of IgM results was inconsistent, suggestive of false positive test results.

Aims/Methods

We set out to determine if any ALFSG patients with indeterminate ALF, presumed DILI, autoimmune hepatitis, hepatitis B, pregnancy, or acetaminophen overdose controls had evidence of acute HEV infection defined as anti-HEV IgM+ or HEV-RNA+. We also set out to determine the seroprevalence of prior HEV infection defined as anti-HEV IgG+ using a previously described ELISA assay (5).

Results

Acute HEV Infection: Three of the 699 (0.4%) patients tested had detectable anti-HEV IgM, and two of these had a high titer anti-HEV IgG consistent with acute HEV infection. However, serum HEV RNA was not detected in these three patients. The three patients were seen in 2004, 2006, and 2008 at study sites in Pennsylvania. All of the subjects were non-Hispanic Caucasian men, and none of them had underlying HIV infection or were receiving immunosuppression. Two of the men aged 36 and 39 were initially suspected of having acute HBV infection, and one of them died from multi-organ failure while another underwent liver transplantation at study day #2 and remains alive and well at 2 years. The third subject was a 71-year-old man with suspected atorvastatin DILI who died of multiorgan failure.

Prior HEV Infection: 294 (42%) of the patients had anti-HEV IgG +, 389 (55%) were anti-HEV IgG – and 18 had borderline test results that were not included in the analysis. The presenting features and outcomes of the two patient groups can be seen in the Table that follows.

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Subjects enrolled from centers in the southeast United States were less likely to be anti-HEV IgG+ compared to other regions (20.6% vs. 47.6%). However, the frequency of anti-HEV IgG + did not vary by study year or month of enrollment.

Conclusions

Acute HEV infection is a rare cause of ALF in the United States, accounting for 0.4% of adult patients tested in the registry. There are no unique or distinguishing presenting features in these cases. Evidence of prior HEV exposure is common in ALF patients and possibly associated with a poorer prognosis but additional analyses are ongoing. Whether anti-HEV IgG + serostatus is due to true prior infection or passive transfer of antibody from blood transfusions is under further investigation.

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	Anti-HEV IgG (+) N = 294	**Anti-HEV IgG (-) N = 389**
Mean Age (yrs)	44 ± 15	40 ± 14
% Male	33.3%	31.9%
% Caucasian	67.3%	76.6%
% African American	18.7%	16.2%
% Asian	7.1%	4.1%
% Other	6.8%	3.1%
% Hispanic	9.2%	11.8%
ALF Diagnosis		
% Acetaminophen	21.8%	34.4%
% Indeterminate	25.9%	20.8%
% Hepatitis B	15%	10.0%
% Idiosyncratic DILI	22.8%	20.6%
% Pregnant	1.4%	2.1%
% Autoimmune Hepatitis	12.9%	11.8%
% Other	0.3%	0.3%
% Blood Transfusion During ALF	86.1%	72.8%
3 Week Outcomes		
% Alive	62.2%	70.7%
% Died	27.8%	27.8%
% Transplanted	35.4%	29.8%

Hepatitis E After Organ Transplantation

Norah Terrault, M.D.

University of California, San Francisco, California, USA

Purpose of the Study:

To determine the magnitude of the clinical problem of HEV in solid organ transplant recipients through review of the available literature.

Statement of the Methods Used:

PubMed search using mesh terms "HEV, transplantation" to February 1, 2012, with hand search of references for additional studies. Primary endpoints evaluated were prevalence/incidence, natural history and outcome of infection. Summary statistics included when applicable. Controlled studies were limited.

Summary of the Results Presented in Sufficient Detail to Support the Conclusion:

1. Incidence/Prevalence

The overall prevalence of HEV infection after solid organ transplant varied from 1.8% to 11.3% (using serologic and virologic markers to define exposure) (Table 1). The prevalence of chronic HEV infection, defined by persistent viremia, varies from 0 to 6.5% (median 0.8%). Genotype 3 was the only genotype reported. Zoonotic transmission is suggested by the findings of two studies. In a case-control study of solid organ transplant recipients in France, ingestion of game meat such as wild boar, deer, and hare was linked with HEV infection in multivariate analysis (OR 2.32, 95% CI1.04-5.15). In a study of pediatric liver transplant recipients from Canada, living in a semi-rural area was reported to be frequent among infected cases. In one case report, the donor of a liver transplant was identified as the likely source of HEV infection.

2. Natural History

Longitudinal studies of HEV in solid organ transplant recipients report that acute hepatitis is characterized by modest ALT/AST elevation (median ALT ~150 U/L, range 0.5 to 26 ULN), with bilirubin elevations in 25%. No case of fulminant liver failure has been reported. Spontaneous resolution of acute infection occurred in ~40% of cases, with resolution more frequent among those infected later after transplantation. Viral clearance is not consistently associated with development of anti-HEV IgG, thus the true incidence of post-transplant HEV infections may be underestimated. HEV reactivation after HEV clearance has not been seen. For those with chronic HEV infection, histological progression to cirrhosis can occur, within 2-3 years in some cases. There are no unique histological findings associated with HEV (versus other viral etiologies of hepatitis) post-transplantation.

3. Treatment and Prevention

Reduction in immunosuppression is advised as the first step in management of chronic hepatitis E in transplant recipients. The benefits of this "intervention" have not been established in any controlled studies. Several case series report that ribavirin (N = 6 treated) or peginterferon (N = 7 treated) therapy is efficacious. Although uncontrolled, the rapid decline in HEV RNA levels within weeks of initiation of drug treatment is consistent with an antiviral drug effect. Treatment courses as short as 3 months have yielded sustained viral clearance in the majority of patients. Given the concern for peginterferon-associated rejection, especially in non-liver solid organ transplant recipients, ribavirin may be the preferred therapy. A vaccine is available but a recommendation for vaccination in solid organ transplant recipients is not justifiable based on the current data.

Statement of the Conclusions:

1. Solid organ transplant recipients are at risk for acute

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and chronic HEV. The overall prevalence of chronic infection, defined by persistent viremia, is low (median 0.8%: range, 0-6.5%). The most frequently identified risk factors include contact with or ingestion of infected game or domestic meat.

2. Spontaneous clearance occurs in ~40% of infected transplant recipients. Those with chronic HEV infection are at risk for chronic and progressive hepatitis, including cirrhosis. Solid organ transplant recipients with persistently elevated ALT or histological hepatitis that are negative for other causes of hepatitis should be tested for HEV infection.

3. Reactivation of HEV infection in persons previously exposed (anti-HEV IgG) does not occur. Thus, patients who are anti-HEV IgG positive before solid organ trasnplantation do not require specific monitoring after transplant.

4. For patients with chronic infection, treatment with short-courses (3 months) of ribavirin or peginterferon monotherapy has been associated with high rates of viral eradication.

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Table 1: Incidence and Prevalence Studies

Author, Country	Study Population	Selection Criteria for Testing	Screening Test Used	Prevalence	Incidence
Kumar (France)	241 kidney 86 liver	Elevation of ALT	IgG/M → if + then HEV RNA HEV RNA if ALT elevation	**6.5%** HEV RNA +	
Legrande (France)	376 kidney 123 liver	No clinical criteria	IgG/M all HEV RNA if Ig+ or ALT elevation	--	3.2/100 p-y 4.8 /100 p-y LT 2.7/100 p-y KT
Buti (Spain)	82 liver 21 kidney 5 liver-kidney	ALT ≥1.5 ULN	Anti-HEV IgG → if + then IgM and HEV RNA	**2.7%** IgG (all liver) All IgM /RNA negative	
Haagsma (Netherlands)	285 liver	No clinical criteria	Anti-HEV IgG and IgM and HEV RNA	**1.8%** any marker **0.07%** IgM ± IgG + **0.35%** HEV RNA only	
Halac (Canada)	80 liver (pediatric)	No clinical criteria	HEV RNA → Anti-HEV IgG/M	**11.3%** IgM and IgG **1.3%** IgM/IgG and RNA	
Pischke (Germany)	226 liver	No clinical criteria	HEV RNA → anti-HEV IgG/M	**1.8%** HEV any marker **0.8%** anti-IgG+ **0.8%** HEV RNA and IgM ± IgG+	

Hepatitis E Infection in HIV-infected Patients

Caryn G. Morse, M.D., M.P.H.

Warren G. Magnuson Clinical Center (CC), National Institutes of Health (NIH), Bethesda, MD

Hepatitis E virus (HEV) is a significant cause of viral hepatitis worldwide. Initially believed to cause only acute infections, recent reports have identified chronic HEV infection in solid organ transplant recipients receiving immunosuppressive therapy and reactivation of hepatitis E infection in immunosuppressed cancer patients. Subsequently, chronic HEV infections in HIV-infected patients have been reported (1,2), suggesting the potential for increased susceptibility to both acute and chronic HEV infection in HIV-infected patients. However, case series in HIV-infected cohorts have found that chronic HEV infections are rare, and there are no data to suggest that HIV-infected individuals are at increased risk of HEV infection compared with the general population (3-7).

Because a diagnosis of HEV infection could reduce the need for further diagnostic testing and potentially avoid antiretroviral therapy change due to concern for drug induced liver injury, evaluation for HEV infection should be considered in HIV-infected patients with unexplained liver enzyme elevations. Since serologic testing may be negative despite ongoing HEV infection (2), PCR testing for HEV RNA is recommended.

Further studies are needed to evaluate the performance of commercial HEV serological assays in patients infected with HIV and to assess the incidence, prevalence, and natural history of HEV infection in HIV-infected patients. Given the global burden of HIV and HEV, research to understand the frequency and clinical consequences of co-infection with these two viruses should be a priority.

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An Assessment of HEV Prevalence and Risk in U.S. Blood Donors and Recipients

Chenyu Xu, Ph.D.; and Harvey J. Alter, M.D., M.A.C.P.

Department of Transfusion Medicine, National Institutes of Health (NIH), Bethesda, Maryland, USA

Hepatitis E virus (HEV) is one of the major causes of acute hepatitis in worldwide. It is now recognized that HEV infection in industrialized countries is mainly autochthonous and has higher prevalence than previously considered. Sporadic cases report serious consequences in immunocompromised patients receiving blood or components that contain HEV RNA. This evidence suggests that the risk of HEV to blood safety may be underestimated. In this study, our aim was to estimate the potential risk of HEV transmission through the U.S. blood supply. 1,050 samples from unselected blood donors were tested for anti-HEV IgG/IgM by ELISA. Sequentially, all anti-HEV antibody positive samples were tested for HEV RNA by nested PCR and quantitative real-time PCR based on the open reading frame 2 region of HEV genome. Prospectively obtained pre- and post- transfusion samples from recipients were tested for HEV antibody and RNA and where indicated, linked donor samples were also tested.

The seroprevalence of anti-HEV IgG in donors was 22.3% (95% confidence interval [CI], 19.8%-24.8%). There was a significant ($P < 0.001$) stepwise increase in prevalence was seen with increasing age, but no association with gender or race. Three of the 1,050 donations were anti-HEV IgM positive. All donations were HEV RNA negative. The prevalence of anti-HEV in the recipient population was 22.7% (95% confidence interval [CI], 13.0%-32.4%) based on pre-transfusion sample testing. Among the 75 recipients tested thus far, there has been no evidence for anti-HEV seroconversion or the appearance of HEV RNA.

These findings indicate that U.S. blood donors frequently have evidence of prior HEV infection. Approximately one-quarter of U.S. volunteer donors tested had evidence of prior exposure to HEV. These donors did not have a history of hepatitis or travel to HEV endemic areas. There is a striking correlation between antibody prevalence and age, suggesting that HEV is a cumulative infection rather than a neonatal transmission of time-defined cohort. In this small sampling, there was no evidence for active viremia, but 0.3% of donors had 1gM anti-HEV, indicating recent exposure. No recipient transmissions were observed, but much larger sampling is required to assess HEV risk in the U.S. blood supply.

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HEV and Food Safety Issues — By Land and Sea to Our Dinner Table

Julie Ann Kase, Ph.D.

U.S. Food and Drug Administration (FDA), Silver Spring, Maryland, USA

Recent published findings and cases of Hepatitis E have prompted a closer examination of what role the hepatitis E virus (HEV) plays in causing foodborne illness. Animal HEV strains have been found in many species, possibly consumed as food, raising public health concerns. Genotype 3 and 4 HEV strains, largely accepted as being the zoonotic genotypes of HEV, have been found in domestic and wild swine, rabbits, and deer. Farm-raised and wild deer have been traditionally hunted in the United States and consumed as game meat. A genotype 3 HEV strain was recovered from sika deer in Japan and roe deer in Hungary; however, feral sika deer in Maryland and Virginia tested negative for anti-HEV antibodies as did caribou from Canada. Red deer, who have a wider geographical range, have tested positive for anti-HEV antibodies. Importantly, findings in Japan have linked the consumption of raw deer meat to HEV infections in humans. A zoonotic link between rabbit HEV and humans still needs to be established. Limited findings have revealed a related but distinct genotype 3 virus in rabbits from China and the United States (Virginia).

Cattle and sheep have been found to be IgG anti-HEV positive. However, virus isolation for confirmation and sequencing has been limited to a single instance with a short PCR amplicon and proof of genotype identity and zoonotic status is purely speculation until further studies are completed. While avian HEV is common in U.S. chicken flocks, it shares little genetic homology (~60%) with either human or swine HEV and has not been demonstrated to infect mammals.

HEV is endemic in domesticated (farmed) swine across the United States. Swine are now acknowledged to be a reservoir for HEV, and cross-species infection has been demonstrated between swine HEV and non-human primates. While cooking (heating to 145°F for whole cuts or 160°F for ground pork) should be enough to inactivate any virus present in pork or pork products, raw or undercooked products pose a palpable risk to human health. Indeed, a survey of grocery stores in Virginia revealed 11% of pig livers tested contained infectious HEV RNA. Furthermore, experimental evidence exists suggesting that the foodborne virus is not as heat labile as previously thought. Overall, further studies are needed to establish viral load in swine pre-slaughter and in various pork products available at market level besides liver since HEV replication may extend beyond hepatic tissues.

In the United States, wild (feral) swine are being hunted, and the meat consumed in ever increasing quantity. While Japan, Australia, and several European countries have documented HEV in feral swine, similar studies have not been published in the United States. Given the high level of seropositivity (up to 42%) and virus recoverability (up to 25%) noted and the recoveries of only genotypes 3 and 4 from feral swine, public health concerns are warranted. Moreover, a nearly genetically indistinguishable HEV strain isolated in Japan was shared between a sika deer, a feral boar, and four humans. An underappreciated threat posed by feral swine in the United States was recently illustrated with the nationwide outbreak of *E. coli* O157:H7 traced to bagged baby spinach grown in California. Ultimately, the source of the O157:H7 contamination was never conclusively identified; however, evidence of field intrusion of feral swine was found, and captured swine tested positive for the bacteria. As the study authors point out, the use of mower-like machines to harvest the product provides ample opportunity for feces to be picked up and contamination to spread widely. If excreted HEV were present, there is no indication the outcome would be any different.

The extent to which HEV may be present in the fecal waste generated from intensive swine agriculture

HEV and Food Safety Issues — By Land and Sea to Our Dinner Table

CONTINUED

operations, as practiced in the United States, is largely unknown and unquantified. One study focused on farms in North Carolina found HEV in stored liquid waste across different seasons and in waste that underwent a secondary stage of treatment. Typically, liquid swine waste does not undergo extensive treatment as is prescribed for human waste. Hence, potentially little or no reduction of fecally shed HEV may occur during swine waste storage before the liquid waste is land applied or used perhaps, as nutrients for greenhouse-grown fruits and vegetables. If infectious HEV is still present in land-applied swine waste, there is the potential for it to be transported off-farm via contaminated surface water, infiltrated groundwater, or irrigated crops.

Another public health concern is agricultural runoff reaching drinking water or shellfish harvesting waters. Bivalve mollusks, such as oysters and clams, feed by allowing water to pass over their gills where particulates, including any pathogenic microorganisms present, are captured and concentrated. Evidence exists implicating shellfish as a foodborne source of infection for two of the eight cases of hepatitis E identified in the United Kingdom in 2005 and an outbreak of Genotype 3 among 33 cruise ship patrons identified the consumption of shellfish as a risk factor. Oyster, in particular, are often consumed raw and are therefore a risky food commodity in terms of human illness. Moreover, a recent autochthonous case of HEV in Texas was linked to the ingestion of raw oysters in 2009. An important agricultural commodity, the latest available figures from the National Marine Fisheries Service cite 35.6 million pounds of oysters were harvested in 2009.

HEV deserves attention as a cause of foodborne illness in the United States. The endemicity of HEV in the domestic and possibly the feral swine population can impact human health through the consumption of pork, pork products, and agricultural contamination of produce and shellfish growing waters with fecal waste. Research findings continue to validate concerns over human exposure risk.

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HEV Vaccine Strategies: Utility and Prospect

J. Wai Kuo Shih, Ph.D.; Jun Zhang; Shao-Wei Li; Ting Wu; Mun-Hon Ng; Qin Jian Zhao; and Ning-Shao Xia

National Institute of Diagnostics and Vaccine Development, School of Public Health, Xiamen University, Xaimen, China

The hepatitis E vaccine, HEV239 (Hecolin), will be commercially available in China in 2012. The vaccine has been shown in a large Phase III clinical trial to be well tolerated by adults and confer 100% protection against hepatitis E caused by both genotypes 1 and 4. Protective immunity was found to develop after two vaccine doses given 1 month apart and also under less than ideal vaccination conditions. Extended analysis of data arising from the study showed the vaccine affords 80% protection against HEV infection and suggests that the vaccine is safe for pregnant women. These findings show that HEV239 vaccine cross protects against infection and disease caused by HEV genotypes 1 and 4 and could be used to prevent and interrupt outbreaks of waterborne infection caused by genotype 1, and possibly genotype 2 virus, in developing countries and to protect travelers to these countries. HEV, mainly genotypes 3 and 4, is prevalent in most developed countries, with HEV sero-prevalence ranging up to more than 50% in some of the areas. Presumably caused through low-level exposure to the virus in the environment (probably water), most of the infection is subclinical, whereas symptomatic disease is rare and generally believed to be mainly caused by food-borne infection. Although uncommon, HEV has emerged to be the principle cause of viral hepatitis and the most severe form of acute hepatitis in many developed countries. It is possible that the vaccine might be beneficial for the elderly populations, who are at high risk of infection, in some of these countries. Further clinical trials are being initiated to determine efficacy and of the vaccine against genotypes 2 and 3 and for high risk groups, such as pregnant women and subjects with chronic liver diseases.

Treatment of Hepatitis E Virus Infection

Nassim Kamar, M.D., Ph.D.

Department of Nephrology, Dialysis and Organ Transplantation, Center Hospitalier Universitaire Rangueil, Toulouse, France

Interest in hepatitis E virus (HEV) infection has re-emerged over the last couple of years with reports of cases of chronic HEV infection occurring in immunosuppressed patients. Indeed, it has been shown that HEV infection may lead to chronic hepatitis in solid-organ-transplant patients, HIV-positive patients, and hematology patients receiving chemotherapy. In solid-organ-transplant and HIV-positive patients, HEV-induced cirrhosis also has been reported. Serial liver biopsies performed in solid-organ-transplant patients have revealed rapid progression of liver fibrosis. In addition, HEV-related extra-hepatic manifestations, such as neurological symptoms and kidney injuries, have been described in immunosuppressed and non-immunosuppressed patients. Hence, because of the possible evolution of HEV infection into chronic hepatitis, physicians have been prompted to set aup strategies for treating
HEV infection.

The reduction of immunosuppressive-drug doses, mainly when they target T-cells, has been shown to achieve HEV clearance in patients with chronic HEV infection. In a large cohort of transplant-patients, 32.1% of patients with chronic hepatitis were cleared of the virus after reducing the immunosuppressant dose. These clinical observations are in line with recent data, which show that HEV-specific T-cell proliferative responses are decreased in transplant patients, particularly in those with chronic hepatitis. Hence, reducing immunosuppressive therapy may be considered a first-line therapeutic option. In solid-organ-transplant patients with chronic hepatitis that remained viremic despite reducing immunosuppressive therapy, anti-viral therapies, namely peginterferon and ribavirin, have been prescribed. A few liver-transplant patients with chronic hepatitis have been given a 3- or 12-month course of peginterferon. A sustained virological response was observed in the majority of these patients. A hemodialysis patient with chronic HEV infection and a previous recipient of a kidney-transplant, but who was not cleared of the virus after immunosuppressant withdrawal and was a candidate for kidney re-transplantation, received a 3-month course of peginterferon alfa 2a (135 μg/week); this resulted in a sustained virological response. Because interferon therapy is contraindicated for kidney-transplant patients and has been associated with a high risk of acute kidney failure, ribavirin has been successfully given to kidney-, kidney-pancreas, and heart-transplant patients with chronic HEV infection. Interestingly, kidney-transplant patients infected with HEV, who are then cleared of the virus and have sustained clearance—i.e., HEV clearance for at least 6 months—do not relapse after re-transplantation despite receiving an immunosuppressive regimen that includes an induction therapy. Hence, dialysis patients previously infected by HEV can be proposed for re-transplantation.

Few data regarding the management of chronic HEV infection in HIV-patients and hematological patients exist. Both peginterferon and ribavirin given as monotherapies, and combined peginterferon plus ribavirin therapy, have been used successfully in this setting.

Fulminant HEV infections have been reported in patients with chronic liver disease whatever its cause. In the setting of "acute on chronic hepatitis," a short course of ribavirin has been shown to achieve complete recovery and avoids the need for liver transplantation. A short course of ribavirin was also given to a non-immunocompromised patient with severe hepatitis and no history of chronic hepatitis; a negative HEV RNA status was reached after 15 days.

In summary, reducing immunosuppressive therapy and ribavirin therapy can efficiently treat chronic HEV infection. Further studies are required to confirm these observations, to determine the optimal length of ribavirin therapy, and to ascertain the factors that offer a sustained virological response.

Poster Abstracts

Identification of a Rabbit Strain of Hepatitis E Virus in the United States and Cross-Species Infection of Pigs

C. Cossaboom[1], L. Córdoba[1], B. Sanford[1], P. Piñeyro[1], S.P. Kenney[1], B.A. Dryman[1], Y. Wang[2], and X.J. Meng[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA; [2]National Institutes for Food and Drug Control, Beijing, China

Hepatitis E virus (HEV) is an important human pathogen. In addition to humans, strains of HEV have also been genetically identified in various other animal species. There are currently four recognized and two putative genotypes of mammalian HEV: genotypes 1 and 2 are restricted to humans while genotypes 3 and 4 are zoonotic, and two putative new genotypes were recently identified from rats and wild boars. HEV is considered as a public health concern in many developing countries, although sporadic cases of acute hepatitis E have also been reported in many industrialized countries, including the United States.

A novel strain of HEV was recently identified from farmed rabbits in China, although its prevalence in other regions is unknown. In an attempt to identify HEV strains from farmed rabbits in the United States, we collected fecal swabs and serum samples from 85 rabbits on two rabbit farms in Virginia. Serum samples were tested for IgG anti-HEV by ELISA, and both fecal swabs and serum samples were tested for HEV RNA by RT-PCR. The prevalence of IgG anti-HEV in rabbits was 36.5% (31/85 rabbits) and HEV RNA was detected in 22% (19/85) rabbits. Sequence and phylogenetic analyses revealed that the U.S. strain of rabbit HEV shared 87.2-89.0% nucleotide sequence identity with the Chinese strains of rabbit HEV and has the highest sequence identity (80.1-82.3%) to the known strains of genotype 3 HEV than other genotypes. Therefore, the rabbit HEV is a distant member of the genotype 3 HEV.
Since pigs are the natural host of genotype 3 HEV, we therefore conducted experiments to determine if the rabbit HEV is antigenically related to other known HEV strains and if it can cross species barriers and infect pigs. We first expressed and purified the recombinant capsid protein of rabbit HEV and showed that the capsid protein of rabbit HEV cross-reacted with antibodies against avian, rat, swine, and human HEVs. Conversely, we also showed that antibodies against rabbit HEV cross-reacted with capsid proteins derived from chicken, rat, swine, and human HEVs. To test if pigs are susceptible to infection by the rabbit HEV, a total of 20 pigs were divided into five groups of 4 pigs each, and intravenously inoculated with PBS (negative control), U.S. rabbit HEV, Chinese rabbit HEV, U.S. rat HEV, and genotype 3 swine HEV (positive control), respectively. Weekly serum and fecal samples from the inoculated pigs were tested for evidence of HEV infection (antibodies by ELISA and viral RNA by RT-PCR). The results showed that, like pigs inoculated with the genotype 3 swine HEV, some pigs inoculated with the rabbit HEV also developed viremia and fecal virus shedding, indicative of active HEV infection. Infection of pigs by rabbit HEV was further verified by demonstration of successful transmission of the virus recovered from pig feces to naïve rabbits. However, the pigs inoculated with a U.S. strain of rat HEV showed no evidence of virus infection.

In summary, in this study we genetically identified and characterized the first U.S. strains of HEV from farmed rabbits and showed that the rabbit HEV belongs to the zoonotic genotype 3. Our results also indicate that rabbit HEV is antigenically related to other known HEV strains and can indeed infect across species barriers. This raises a concern of potential zoonotic transmission of the rabbit HEV from rabbits to humans through direct contact with infected rabbits or through the consumption of undercooked rabbit meat.

Detection of Hepatitis E Virus in Water Proximal to Swine Concentrated Animal Feeding Operations in Eastern North Carolina

Jennifer Gentry-Shields, M.S.[1]; Chris Heaney, Ph.D.[2]; Kevin Myers, M.S.[3]; Devon Hall, Ph.D.[1]; Dothula Baron-Hall, Ph.D.[1]; Steve Wing, Ph.D.[1]; and Jill Stewart, Ph.D.[1]

[1]The University of North Carolina, Chapel Hill, North Carolina, USA; [2]The Johns Hopkins University, Baltimore, Maryland, USA; [3]Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The number of Concentrated Animal Feeding Operations (CAFOs) in the United States has increased dramatically in the last few decades. In North Carolina, the majority of the hog inventory is now raised in CAFOs. Rapid growth of the hog CAFO industry has raised concern about proper disposal of waste, which in North Carolina is typically collected and stored in open-pit lagoons before being sprayed onto agricultural fields. Components of this waste may be able to impact surface water quality with the potential for human health risks. This study examined creeks adjacent to swine CAFO spray fields for the presence of hepatitis E virus (HEV). Surface water samples were collected from public access waters in proximity to spray fields over 1 year and were tested for HEV using end-point real-time polymerase chain reaction (PCR) and coliphages using standard culture-based methods. HEV was detected in one sample in March 2010. This water sample also contained levels of somatic coliphages, fecal coliforms, *E. coli*, and *Enterococcus* sp. that were too numerous to count (TNTC). Somatic and F+ coliphages, indicators of enteric viruses, were detected in 98% and 85% of samples, respectively. The presence of HEV, as well as the ubiquity of coliphages, suggests that HEV from swine waste may be able to contaminate proximal surface waters from swine CAFO spray fields.

A Cross Sectional Study of Hepatitis E Virus Infection Among Pig Farmers and Healthy Controls Without Direct Pig Exposure in Rural Northern Thailand

Soawapak Hinjoy, D.V.M., Dr.P.H.[1,2]; Kenrad E. Nelson, M.D.[1]; Robert V. Gibbons, M.D.[3]; Richard G. Jarman, Ph.D.[3]; Duangrat Mongkolsirichaikul, Ph.D.[3]; Punnarai Smithsuwan, D.V.M.[2]; and Alain Labrique, Ph.D.[1]

[1]Department of Epidemiology, The Johns Hopkins University School of Public Health, Baltimore, Maryland, USA; [2]Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand; [3]Armed Forces Research Institute for Medical Sciences (AFRIMS), Bangkok, Thailand

We conducted a cross-sectional study of the association between occupational pig exposure and HEV infection in adult pig farmers from three sizes of farms—i.e., small (< 30 pigs), medium (30-100 pigs), and large (> 100 pigs)—in Nan Province in rural Northern Thailand. We selected 171 pig farmers from a provincial census of pig farms. They were matched by age and sex with persons not having direct contact with pigs who were living in Nan Province. All subjects were interviewed about their job history, exposure to pigs, eating habits, hygiene history, and environmental exposures. The anti-HEV IgG seroprevalence among the 513 study subjects was 23.0%, using a research EIA assay developed by the AFRIMS laboratory in Bangkok. The anti-HEV IgG prevalence in pigs was 9.9%. Sera from 20 pigs were HEV RNA positive for genotype 3 strains. The seroprevalence was not different between farmers and non-farmers. However, among swine farmers, handwashing with soap versus water was protective and flooding of the house and farm during the rainy season was a significant risk factor. The strongest risk factor for HEV seroprevalence was consumption of internal pig organs more than twice a week: OR = 3.23 (95% CI 1.15-9.01). Among both farmers and non-farmers consumption of pig liver, sometimes covered with pig blood, a Thai dish called "Larb" (pig liver) or "Larb loo" (pig liver with blood), was a very common delicacy. Only 14% of subjects reported that they "Never" ate Larb and 10% ate Larb at least twice per week, on average. Most subjects (77%) reported eating Larb once a week or less, but frequently.

In this population, dietary consumption of raw pig liver was a greater risk for HEV seroprevalence than direct pig contact. However, among farmers, handwashing with soap was protective, and flooding was a significant risk for HEV seroprevalence. These data suggest that dietary exposures to HEV from pig liver consumption overwhelmed the more moderately increased risk of infection from direct contact with pigs in this population.

Inhibition of Hepatitis E Virus Replication by Proteasome Inhibitor MG132

Yogesh A. Karpe and Xiang-Jin Meng

Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Hepatitis E virus (HEV), a non-enveloped single-strand positive-sense RNA virus in the family *Hepeviridae*, is an important but understudied human pathogen. The mechanism of HEV replication remains largely unknown. The cellular ubiquitin-proteasome system (UPS) is important for intracellular protein degradation in eukaryotic cells and plays a central role in cellular protein homeostasis. UPS has been implicated in the infection cycle and virus-host interplay of several viruses.

In this study, we tested the effect of proteasome inhibitors MG132, Lactacystin and Epoxomicin on HEV replication. Initially, we used HEV replicon expressing *Renilla* luciferase gene, to study the effect of these inhibitors on HEV replication. All the inhibitors tested in this study caused a significant reduction in the level of virus replication. The MG132 has a more pronounced effect on virus replication than other inhibitors. Furthermore, we demonstrated that this inhibition of HEV replication is concentration-dependent. Results were further confirmed by immunofluorescence assay and negative strand-specific RT-PCR. We subsequently tested the effect of the inhibitor treatment on GFP synthesis. In addition, in another set of experiments, we expressed part of a viral capsid protein that is known to form virus like particles (VLPs) in the presence and absence of MG132. Comparable levels of GFP, capsid protein, and actin were observed in drug-treated and untreated cells. These results strongly suggest that there is an inhibition of viral replication without significant effect on cellular translation.

Results from this study suggest that HEV replication requires an active ubiquitin-proteasome system, and that proteasome inhibitors affect HEV replication possibly by inhibition of viral transcription or/and translation without significant effect on cellular translation. Overexpression of ubiquitin in inhibitor-treated cells partially reverses the inhibitor effect on HEV replication. In conclusion, HEV replication requires interactions with proteasome machinery that could be a potential therapeutic target against HEV.

Seroincidence of Intrapartum Hepatitis E Virus Infections and Micronutrient Status in Pregnant Bangladeshi Women Between 2001 and 2010

Alain B. Labrique, Ph.D., M.H.S., M.S.[1]; Sabra Klein, Ph.D., M.S., M.A.[1]; Brittany Kmush, Sc.M.[1]; Wei Li, Ph.D.[1]; Hasmot Ali, M.B.B.S., M.P.H.[2]; Ronald E. Engle[3]; Kerry Schulze, Ph.D., M.S.[1]; Robert H. Purcell, M.D.[3]; and Kenrad E. Nelson, M.D.[1]

[1]The Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]The JiVitA Maternal and Child Health Research Project, Gaibandha, Bangladesh; [3]National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA

Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis globally and results in severe morbidity and mortality in pregnant women. There is a paucity of longitudinal data examining the incidence and disease rate of HEV in cohorts of pregnancy in endemic areas. We studied serial sera collected within two prospective cohorts totaling 110,473 incident pregnancies enrolled in large randomized trials in rural northwestern Bangladesh, between 2001–2006 (cohort A) and 2008–2010 (cohort B). An NIH research immunoassay was used to identify anti-HEV IgG status in early pregnancy, late pregnancy, and 3-month postpartum venous blood specimens, drawn on a subsample of the larger cohorts. Of the 1,127 specimens available for testing in cohort A, 72 were anti-HEV seropositive at baseline, indicating a seroprevalence of ~6.4%. During this period, 63 women were identified as potential seroconverters, suggesting an incidence rate of ~56 infections per 1,000 person-years. In the more recent cohort B, 1,100 were available for testing, revealing a ~6.1% seroprevalence in anti-HEV IgG at early pregnancy. Within this cohort, 40 women were identified as putative seroconverters, an incidence rate of 46 infections per 1,000 person-years. Between the 2001–2006 cohort and the 2008–2010 cohort, the incidence of intrapartum HEV infections seems to be declining in rural Bangladesh, possibly reflective of improved sanitation.

Micronutrient and cytokine analysis of the 2008–2010 cohort is ongoing to characterize the immunopathology of HEV infection. Initial data suggest that seroconverters seem to have lower baseline micronutrient concentrations compared to the controls. Zinc concentrations were significantly lower in seroconverters than in controls (749 ± 133 versus 814 ± 151 μg/L, $p = 0.04$). Seroconverters had three times greater prevalence of anemia as measured by circulating hemoglobin than controls. However, concentrations of plasma ferritin, a marker of iron status, were normal in this population; only 2.5% of seroconverters and 5.0% of controls had inadequate concentrations ($p = 0.56$). Vitamin D deficiency was very prevalent as 95.0% of cases and 82.5% of controls ($p = 0.08$) had concentrations of 25-hydroxy-vitamin D concentrations below 50 nmol/L. Additionally, the prevalence of inflammation (C - reactive protein) was similar early in pregnancy between the cases and controls. These differences in micronutrient status were all observed early in pregnancy. Therefore, women who became infected with HEV later in pregnancy began their pregnancies with a different micronutrient profile that the women who did not become infected. Additionally, the seroconverters had higher levels of both pro- and anti-inflammatory cytokines, suggesting general immune dysregulation. These data suggest that micronutrient deficiencies in early pregnancy may predispose women to subsequent HEV infection.

Severe, Fatal Chronic Hepatitis E Post-liver Transplant

Vinaya C. Maddukuri[1]; Mark W. Russo[1]; William A. Ahrens[1]; Suzanne U. Emerson[2]; Ronald E. Engle[2]; Robert H. Purcell[2]; Eric B. Thompson[3]; and Herbert L. Bonkovsky[1]

[1]The Liver-Biliary-Pancreatic Center and Liver Transplant Center, Carolinas Medical Center, Charlotte, North Carolina, USA; [2]Molecular Hepatitis and Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; [3]Carolinas Digestive Health Associates, Charlotte, North Carolina, USA

Hepatitis E virus (HEV) was previously thought to cause an acute self-limiting illness. However, HEV has recently been recognized to cause chronic infection in immunocompromised hosts like HIV infected patients, patients receiving anti-cancer chemotherapy, and solid organ transplant recipients [1]. Here, we describe the clinical, histopathological, and virological features of a carefully studied subject who developed acute HEV (hepatitis E virus) infection following a liver transplant. The HEV infection became chronic and led to rapidly progressive liver injury and to development of central nervous system (CNS) infection and complications. We summarized clinical features from medical records; histopathology of repeated liver biopsies was assessed by W. Ahrens, an experienced hepatopathologist; HEV virological studies were performed by S. Emerson, R. Engle, and R. Purcell (NIAID/NIH).

Summary of Case: A 64-year-old white man underwent orthotopic liver transplantation for decompensated cirrhosis due to alcohol. The donor was a 51-year-old man with no preexisting liver disease who died due to closed head injury. Histology of the donor liver showed 10-20% macrovesicular steatosis but no other abnormality. After liver transplantation, our patient was treated with usual doses of corticosteroids and tacrolimus for immunosuppression with a trough tacrolimus goal of 12-15 ng/mL. However, multiple doses of anti-thymocyte globulin (OKT-3) and additional steroid boluses were administered in the second week after transplant for treatment of severe acute cellular rejection (rejection activity index 8 of 9). His eventual discharge immunosuppressive regimen after transplant consisted of tacrolimus, mycophenolate mofetil, and steroids. The rejection resolved and his liver tests were completely normal 1 month after transplant. A year after the transplant, the man developed chronic persistent low-grade elevations of serum aminotransferases, associated with poor appetite, weight loss, and severe muscle wasting. He complained of generalized weakness, difficulty concentrating, memory loss, dizziness, problems with balance, frequent falls, urinary retention, and paraesthesias of hands and feet. Serologic workup for hepatitis A, B, and C was negative. Liver biopsy was essentially unremarkable without any evidence of viral hepatitis, CMV, rejection, or drug-induced liver injury. However, subsequent biopsies, performed 2 and 3 years post-transplant, showed lymphohistiocytic portal and lobular inflammation and acidophil necrosis consistent with viral hepatitis. There was clear evidence of rapid progression of fibrosis on liver biopsies from stage 0 to stage 3 over a 2-year period. Tests for HEV infection were performed 40 months after transplant. The results showed anti-HEV IgM, IgG, and HEV RNA in serum. The genotype of the HEV was 3. Detailed sequencing of the HEV genome isolated from our patient showed a result similar to that of the previously isolated Kernow C1 strain, which was responsible for causing chronic hepatitis E in an HIV patient (2). Specifically, the quasispecies of both strains included a human ribosomal protein gene sequence insertion into the hypervariable region (HVR) of the HEV open reading frame-1(2).

Our patient was started on Peg-IFN-alpha 2a [90 mcg/wk] for HEV while maintaining minimal immunosuppression with tacrolimus (goal trough level of 3-5 ng/mL). The dose was decreased to 45 mcg/wk at week 8, due to worsening fatigue. IFN treatment was discontinued after 15 weeks because of poor patient tolerance and lack of effect on serum ALT or AST levels. He did not receive ribavirin. Repeat testing of sera, urine, stool, and CSF after treatment was positive for HEV RNA. His progressive decline continued, and he died

Severe, Fatal Chronic Hepatitis E Post-liver Transplant

CONTINUED

6 months after stopping interferon treatment and 5 years after transplant. An autopsy was not performed.

We conclude that: 1. HEV is a cause of acute and/or chronic hepatitis among persons with immunosuppression such as persons who have undergone liver transplants. 2. The hepatic histopathology is typical of viral hepatitis A-E. 3. Hepatic fibrosis may progress rapidly. 4. Severe systemic and neurological manifestations may occur with evidence of HEV in the CSF; these may be fatal. 5. Therapy with Peg-IFN-alpha 2a [Pegasys] may not eradicate HEV.

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(2) Nguyen, H.T., et al. A naturally occurring human/hepatitis E recombinant virus predominates in serum but not in faeces of a chronic hepatitis E patient and has a growth advantage in cell culture. *J Gen Virol* 2012; 93(Pt 3): 526-30.

Hepatitis E Genotype 3 Viral Infection in a University Hospital in the Netherlands Over a 5-Year Period

Annelies Riezebos-Brilman, M.D., Ph.D.; and
Hubert G.M. Niesters, Prof. Ph.D.

Department of Medical Microbiology, Division of Clinical Virology, The University of Groningen, University Medical Center Groningen (UMCG), The Netherlands

Hepatitis E virus (HEV) has long been known as a major cause of acute hepatitis in developing countries, with occasional travel-related cases in developed countries. Travel-related HEV is mainly caused by genotype 1 and 2; these are strictly human viruses. Recently, HEV genotype 3 infections are being recognized as a public health issue in developed countries and are believed to be a porcine zoonosis. Although HEV was considered as an agent responsible for an acute hepatitis, increasing information indicates that HEV genotype 3 infection may lead to chronic hepatitis in immunocompromised patients.

The UMCG is a tertiary referral hospital providing care to both adults and children and has a large solid organ transplant program. Since the second half of 2007, HEV diagnostics are performed in patients with an unexplained hepatitis. Serology is being performed with a HEV ELISA, followed by a confirmatory Western immunoblot. HEV RNA is being detected in plasma, serum, or feces using a real-time PCR. Subsequently, the HEV is characterized by sequencing of the ORF1 and ORF2 region.

In total, 26 patients showed to be positive for HEV virus in serum or plasma by PCR. The majority of the patients were immunocompromised, 18 were solid organ transplant (SOT) patients, and 5 were patients immunocompromised for other reasons. Only 3 patients diagnosed with HEV were immunocompetent. A chronic infection could be diagnosed in 13 of these SOT recipients (72%), and one patient died of liver failure. In total, 5 SOT recipients were able to clear the infection after lowering the immunosuppressive medication and 6 were successfully treated with either pegylated interferon alpha-2b or oral ribavirin. Viral genotyping, strikingly, revealed only genotype 3 strains—all swine related.

HEV hepatitis is an underdiagnosed pathogen in immunocompromised patients. Although the general recommendation is to lower the immunosuppressive medication, there is currently no specific treatment option. However, both pegylated interferon alpha-2b and oral ribavirin have been described as potentially effective treatments of HEV infection. Early detection of HEV infection is essential to minimize liver damage and maximize the effect of antiviral therapy. Therefore, an increasing awareness for HEV amongst clinicians and medical microbiologists is needed. Despite the fact that all isolates were pig-related, the origin of the infection remained unclear.

Chronic Hepatitis E Infection in Lung Transplant Recipients

Annelies Riezebos-Brilman, M.D. Ph.D.[1]; Elisabeth Puchhammer-Stöckl, Prof. Ph.D.[4]; Isabella Bejvl[4]; Peter Jaksch, M.D. [5]; Elizabeth B. Haagsma, M.D., Ph.D.[2]; Hubert G.M. Niesters, Prof. Ph.D.[1]; and Erik A.M. Verschuuren, M.D., Ph.D.[3]

[1]Department of Medical Microbiology, Division of Clinical Virology, [2]Department of Gastroenterology and Hepatology, and [3]Department of Pulmonary Diseases, The University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; [4]Department of Virology, and [5]Department of Internal Medicine, Medical University of Vienna, Vienna, Austria

Liver function test abnormalities are very common after solid organ transplantation. There are now increasing numbers of reports showing that hepatitis E virus (HEV) infection may cause a chronic hepatitis in solid-organ transplant recipients. In these patients, chronic infection may lead to rapidly progressive cirrhosis.

To date, there is no specific treatment for HEV infection. For solid organ transplant recipients, the general recommendation is to lower the immunosuppressive medication. Recently, both pegylated interferon alpha-2b and oral ribavirin have been described as potentially effective treatments of HEV infection. Early detection of HEV infection is essential to minimize liver damage and maximize the effect of antiviral therapy.

The objective of the study is to investigate the clinical impact of HEV infection in lung transplant recipients from two university hospitals in two European countries.

The study group consisted of adult lung transplant recipients of two University Medical Centers: the Medical University of Vienna (MUV) and the University Medical Center Groningen (UMCG). Both hospitals are tertiary referral hospitals, providing large solid organ transplant programs. Lung transplant recipients presenting with elevated liver tests (ALT levels > 40) during the post transplant follow-up period were tested for viral hepatitis, including HEV. After identification of HEV as the cause of the hepatitis, freeze-stored samples of serum, plasma, or whole blood were retrospectively tested for presence of HEV RNA. In order to determine the time of infection, HEV RNA is tested in whole blood, plasma, serum, or feces using a real-time polymerase chain reaction (PCR). Subsequently, the HEV is characterized by sequencing of the ORF1 and ORF2 region.

In this study, 202 adult lung transplant recipients of the UMCG and 263 of the MUV were included. In total, 23 of the 202 patients (11%) and 58 of the 263 patients (22%) showed elevated liver tests. Of these patients, four of the study cohort in the UMCG (2% of total) and six of the study cohort in the MUV (2.28%) tested positive for HEV RNA in blood. The HEV positive patients ranged in age from 25 to 68 years, with a median age of 40 years. Males and females were divided 60 to 40%, respectively. At the time of HEV detection, all patients showed elevated liver tests, with ALT levels ranging from 44 to 156 U/L (normal < 30 U/L), with a median of 77 U/L. In all 10 lung transplant recipients, a chronic HEV infection could be confirmed by persistently detectable HEV RNA in blood samples for a period over 6 months. Viral genotyping revealed only genotype 3 strains. The patients were infected with a median of 5.5 months (range 2-106) post-transplant. The hepatitis was mild in all cases. Two of the lung transplant recipients were treated with oral ribavirin monotherapy. A rapid clearance of HEV RNA after 2 months could be observed in both patients, which corresponded with normalized ALT levels.

In conclusion, low-grade liver function test abnormalities after lung transplantation can be caused by chronic HEV infection. The favorable outcome upon antiviral treatment in two lung transplant recipients suggests that oral ribavirin might be an effective treatment of chronic HEV infection in lung transplant patients. Further clinical studies are needed to investigate the effectiveness of antiviral (ribavirin) treatment in different groups of solid organ transplant recipients.

Identification of Hepatitis E Virus Genotype 3 From Wild-collected *Rattus* at Multiple U.S. Localities

Ronald A. Van Den Bussche, Ph.D.; Kylie Volk; and Justin B. Lack, M.S.

Department of Zoology, Oklahoma State University, Stillwater, Oklahoma, USA

The role of rodents in the epidemiology of zoonotic hepatitis E virus (HEV) has been a subject of considerable debate. Seroprevalence studies suggest widespread HEV infection in commensal *Rattus*, but transmissions have been largely unsuccessful, and the recovery of zoonotic genotype 3 HEV RNA from wild *Rattus* has never been confirmed. We surveyed *R. rattus* and *R. norvegicus* from across the United States and several international populations using a hemi-nested RT-PCR approach. We recovered HEV RNA in liver tissue from 35 individuals out of 446 examined. Based on phylogenetic analyses, all but one of these isolates clustered with previously identified zoonotic HEV genotype 3, and the remaining sequence represented the recently discovered rat genotype from the United States and Germany. Positive individuals were detected in both urban (e.g., San Francisco Bay Area, Memphis) and remote (Aleutian Islands) localities. Phylogenetic analyses suggested that all HEV genotype 3 infections we detected in *Rattus* are the result of a single strain.

Participants List

Harvey Alter, M.D.
Chief, Infectious Diseases Section
Associate Director for Research
Department of Transfusion Medicine
National Institutes of Health
Building 10, Room 1-C711
10 Center Drive, MSC 1184
Bethesda, MD 20892-1184
Telephone: (301) 496-8393
Fax: (301) 402-1360
Email: halter@dtm.cc.nih.gov

Howard Anderson, Ph.D.
Division of Therapuetic Proteins
U.S. Food and Drug Administration
29 Lincoln Drive
Bethesda, MD 20892
Telephone: (301) 827-1781
Email: howard.anderson@fda.hhs.gov

Betty Apica, M.D.
Department of Internal Medicine
University of Texas Southwestern Medical Center
P.O. Box 1.04.420
5959 Harry Hines Boulevard
Dallas, TX 75390-8887
Telephone: (214) 645-6111
Fax: (214) 645-6114
Email: betty.apica@utsouthwestern.edu

Irwin Arias, M.D.
Group Leader
Cell Biology and Metabolism Program
Office of Clinical Research Training
and Medical Education
Eunice Kennedy Shriver National Institute
of Child Health and Human Development
National Institutes of Health
Building 32TI, Room 100
9000 Rockville Pike
Bethesda, MD 20814
Telephone: (301) 402-8394
Email: irwin.arias@nih.gov

Ludmila Asher, M.D.
Department of Pathology
Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910
Telephone: (301) 319-9327
Email: ludmila.asher@us.army.mil

Mark Avigan, M.D., C.M.
Office of Surveillance and Epidemiology
U.S. Food and Drug Administration
Building S1, Room 2208
10993 New Hampshire Avenue
Silver Spring, MD 20993
Telephone: (301) 796-0065
Email: mark.avigan@fda.hhs.gov

Patricia Bare, Ph.D.
Visiting Scientist
Department of Transfusion Medicine
National Institutes of Health
Building 10, Room 1C-711
10 Center Drive
Bethesda, MD 20892
Telephone: (301) 496-8393
Fax: (301) 402-2965
Email: pbare@hematologia.anm.edu.ar

Raija Bettauer, J.D.
Consultant
Bettauer BioMed
1518 Highwood Drive
McLean, VA 22101
Telephone: (703) 536-0729
Email: raija.bettauer@verizon.net

Robin Biswas, M.D.
Supervisory Medical Officer
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
HFM-310
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-3011
Fax: (301) 480-7928
Email: robin.biswas@fda.hhs.gov

Participants List

Erich Boger, Ph.D.
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-5157
Email: erich.boger@fda.hhs.gov

Herbert Bonkovsky, M.D.
Professor of Medicine
Departments of Internal Medicine and Research
Carolinas Medical Center
Cannon Research Center
1542 Garden Terrace, Suite 201
Charlotte, NC 28203
Telephone: (704) 355-3959
Fax: (704) 355-7648
Email: herbert.bonkovsky@carolinas.org

Dianjun Cao, Ph.D.
Research Scientist
Department of Biomedical Science
Virginia Polytechnic Institute and State University
1981 Kraft Drive
Blacksburg, VA 24060
Telephone: (540) 577-4973
Email: djcao@vt.edu

Allene Carr-Greer, B.S.
Director
Department of Regulatory Affairs
AABB
8101 Glenbrook Road
Bethesda, MD 20814
Telephone: (919) 658-1689
Email: acarrgreer@aabb.org

Philippe Colson, Ph.D., Pharm.D.
Pole des Maladies Infectieuses et Tropicales Clinique et Biologique, Federation de Bacteriologie-Hygiene-Virologie
Centre Hospitalo-Universitaire Timone, AP-HM
264, rue Saint-Pierre
Marseille 13385
France
Telephone: 33491324375
Email: philippe.colson@univ-amu.fr

Caitlin Cossaboom, B.S.
Department of Biomedical Sciences and Pathobiology
Virginia Polytechnic Institute and State University
1981 Kraft Drive, Room 2036
Blacksburg, VA 24061
Telephone: (540) 231-6912
Fax: (540) 231-3414
Email: cmcossa@vt.edu

Martha Jane Coury, M.S.N.
Program Officer, Middle East/North Africa/Europe
Office of Global Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 6610, Room 5213
6610 Rockledge Drive, MSC 6613
Bethesda, MD 20892-6613
Telephone: (301) 451-3062
Fax: (301) 480-2954
Email: courym@niaid.nih.gov

Harry Dalton, Ph.D.
Cornwall Gastrointestinal Unit
Royal Cornwall Hospital and European Centre for Environment and Human Health
Penventinnie Lane
Truro TR1 3LJ
United Kingdom
Telephone: 447870347122
Email: harry.dalton@rcht.cornwall.nhs.uk

Lu Deng, Ph.D.
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
27 Lincoln Drive
Bethesda, MD 20892
Email: lu.deng@fda.hhs.gov

Edward Doo, M.D.
Program Director
Liver Diseases Research Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 651
6707 Democracy Boulevard, MSC 5450
Bethesda, MD 20892-5450
Telephone: (301) 451-4524
Email: dooe@niddk.nih.gov

Jan Drobeniuc, M.D.
Microbiologist
Division of Viral Hepatitis
Centers for Disease Control and Prevention
1600 Clifton Road, MS A33
Atlanta, GA 30329
Telephone: (404) 639-3790
Fax: (404) 639-1563
Email: jqd6@cdc.gov

D. Robert Dufour, M.D.
Consultant
Department of Hepatology
Veterans Affairs Medical Center
50 Irving Street, N.W.
Washington, DC 20422
Telephone: (240) 432-7760
Fax: (202) 745-8284
Email: chemdoctorbob@earthlink.net

Suzanne Emerson, Ph.D.
Head
Molecular Hepatitis Section
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 50, Room 6537
50 South Drive, MSC-8009
Bethesda, MD 20892-8009
Telephone: (301) 496-2787
Fax: (301) 402-0524
Email: semerson@niaid.nih.gov

Ron Engle, B.S.
Microbiologist
Laboratory of Infectious Diseases
Hepatitus Virus Section
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Twinbrook 1 Facility, Room 1505
5640 Fishers Lane, MSC 8152
Rockville, MD 20852-8152
Telephone: (301) 435-2445
Fax: (301) 480-1280
Email: rengle@niaid.nih.gov

Jay Epstein, M.D.
Director
Office of Blood Research and Review
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-3518
Fax: (301) 827-3533
Email: jay.epstein@fda.hhs.gov

Narnygerel Erdenebileg, M.D.
Visiting Fellow
Infectious Disease and Immunogenetics Section
Department of Transfusion Medicine
National Institutes of Health
Building 10, Room 1C711
10 Center Drive, MSC 1184
Bethesda, MD 20892-1184
Telephone: (301) 451-8619
Fax: (301) 480-9203
Email: erdenebilegn@mail.nih.gov

Peter Evans, Ph.D., M.P.H.
Office of Public Health Science
Food Safety and Inspection Service
U.S. Department of Agriculture
1400 Independence Avenue, S.W.
Washington, DC 20250
Telephone: (202) 690-6128
Fax: (202) 690-6364
Email: peter.evans@fsis.usda.gov

Participants List

James Everhart, M.D., M.P.H.
Medical Officer
Digestive Diseases Programs Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 655
6707 Democracy Boulevard, MSC 5450
Bethesda, MD 20892-5450
Telephone: (301) 594-8878
Fax: (301) 480-8300
E-mail: je17g@nih.gov

Mahmood Farshid, Ph.D.
Regulatory Scientist
Division of Hematology
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
HFM-345, Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448
Telephone: (301) 827-6163
Email: mahmood.farshid@fda.hhs.gov

Robert Fontana, M.D.
Professor of Medicine
Department of Internal Medicine
University of Michigan
3912 Taubman Center
Ann Arbor, MI 48109-0362
Telephone: (734) 936-4780
Fax: (734) 936-7392
Email: rfontana@med.umich.edu

Nancy Fryzek, B.S.N.
Research Nurse Specialist
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 4-5722
10 Center Drive, MSC 1614
Bethesda, MD 20892-1614
Telephone: (301) 435-6122
Email: nancy.fryzek@nih.gov

Guang Gao, Ph.D.
Regulatory Scientist
Product Review Branch
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
HFM 394
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-5116
Email: guang.gao@fda.hhs.gov

Naveen Gara, M.D.
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 4-5722
10 Center Drive, MSC 1800
Bethesda, MD 20892-1800
Telephone: (301) 451-6983
Email: naveen.gara@nih.gov

Marc Ghany, M.D.
Staff Physician
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 9B-16
10 Center Drive, MSC 1800
Bethesda, MD 20892-1800
Telephone: (301) 402-5115
Fax: (301) 402-0491
Email: marcg@intra.niddk.nih.gov

Jonathan Goldsmith, M.D.
Medical Officer
Blood Diseases Branch
Division of Blood Diseases and Resources
National Heart, Lung, and Blood Institute
National Institutes of Health
Two Rockledge Center, Room 9156
6701 Rockledge Drive, MSC 7950
Bethesda, MD 20892-7950
Telephone: (301) 435-0050
Fax: (301) 480-1046
Email: goldsmithjc@nhlbi.nih.gov

Nan Guo, Ph.D.
Department of Epidemiology
The Johns Hopkins University
615 N. Wolfe Street
Baltimore, MD 21210
Telephone: (410) 456-9316
Email: nguo@jhsph.edu

Hwalih Han, B.S.N.
Research Nurse Specialist
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 4-5722
10 Center Drive, MSC 1614
Bethesda, MD 20892-1614
Telephone: (301) 496-1665
Email: hanhl@niddk.nih.gov

Jane Harman, D.V.M., Ph.D.
Division of Cardiovascular Sciences
National Heart, Lung, and Blood Institute
National Institutes of Health
Rockledge Two, Suite 10018
6701 Rockledge Drive, MS 7936
Bethesda, MD 20892-7936
Telephone: (301) 435-1290
Email: jane.harman@nih.gov

Vanessa Haynes-Williams, M.S.N.
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 4-5722
10 Center Drive, MSC 1614
Bethesda, MD 20892-1614
Telephone: (301) 451-7007
Fax: (301) 451-9160
Email: vhaynes@cc.nih.gov

Christopher Heaney, Ph.D.
Assistant Professor
Department of Environmental Health Sciences and Epidemiology
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street, Room W7033B
Baltimore, MD 21205
Telephone: (443) 287-4989
Fax: (443) 287-4989
Email: cheaney@jhsph.edu

Theo Heller, M.D.
Investigator
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 9B16
10 Center Drive, MSC 1800
Bethesda, MD 20892-1800
Telephone: (301) 496-1721
Fax: (301) 402-0491
E-mail: theller@nih.gov

Kaoru Hida, Ph.D.
ORISE Fellow
Center for Food Safety and Applied Nutrition
Office of Applied Research and Safety Assessment
U.S. Food and Drug Administration
Mod I Building
8301 Muirkirk Road
Laurel, MD 20708
Telephone: (301) 796-0601
Email: kaoru.hida@fda.hhs.gov

Kirsten Hirnesen, M.S.
Graduate Student
Department of Animal and Food Sciences
University of Delaware
044 Townsend Hall
531 S. College Avenue
Newark, DE 19716
Telephone: (302) 831-6513
Email: khirneis@udel.edu

Participants List

Sally Hojvat, Ph.D., M.S.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics Safety
and Evaluation
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
Telephone: (301) 796-5455
Email: sally.hojvat@fda.hhs.gov

F. Blaine Hollinger, M.D.
Professor of Medicine, Molecular Virology,
and Epidemiology
Baylor College of Medicine
One Baylor Plaza, BCM-385
Houston, TX 77030
Telephone: (713) 798-3008
Fax: (713) 798-3490
Email: blaineh@bcm.edu

Scott Holmberg, M.D.
Branch Chief
Epidemiology and Surveillance Branch
Division of Viral Hepatitis
National Center for HIV/AIDS, Viral Hepatitis,
STD, and TB Prevention
Centers for Disease Control and Prevention
1600 Clifton Road, N.E., Mailstop G-37
Atlanta, GA 30329
Telephone: (404) 718-8550
Email: sdh1@cdc.gov

Jay Hoofnagle, M.D.
Director
Liver Disease Research Branch
National Institute of Diabetes and Digestive
and Kidney Diseases
National Institutes of Health
Building 31, Room 9A27
31 Center Drive, MSC 2560
Bethesda, MD 20892- 2560
Telephone: (301) 496-1333
Fax: (301) 480-7926
Email: hoofnaglej@extra.niddk.nih.gov

Yaowei Huang, Ph.D.
Research Assistant Professor
College of Veterinary Medicine
Virginia Polytechnic Institute and State University
1981 Kraft Drive
Blacksburg, VA 24061
Telephone: (540) 231-4339
Email: yhuang@vt.edu

Pawan Jain, M.D., Ph.D.
Biologist
Performance Review Board
Division of Emerging and Transfusion
Transmitted Diseases
Center for Biologics Evaluation and Research
Office of Blood Research and Review
U.S. Food and Drug Administration
HFM-394
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-5115
Email: pawan.jain@fda.hhs.gov

Stephen James, M.D.
Director
Division of Digestive Diseases and Nutrition
National Institute of Diabetes and Digestive
and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 677
6707 Democracy Boulevard, MSC 5450
Bethesda, MD 20892-5450
Telephone: (301) 594-7680
Fax: (301) 480-8300
Email: jamess@extra.niddk.nih.gov

Janet Jezsik
Physician Assistant
Division of Gastroenterology
Department of Medicine
Duke University Medical Center
200 Trent Drive
Durham, NC 27704
Telephone: (919) 684-2052
Fax: (919) 684-8857
Email: janet.jezsik@duke.edu

Laure Juompan, Ph.D.
Biologist
Division of Emerging and Transfusion Transmitted Diseases
Office of Blood Research and Review
U.S. Food and Drug Administration
HFM 323, Suite 200N
1401 Rockville Pike
Rockville, MD 20853
Telephone: (301) 594-1137
Email: laure.juompan@fda.hhs.gov

Nassim Kamar, M.D., Ph.D.
Professor
Department of Nephrology, Dialysis, and Organ Transplantation
Toulouse University Hospital
1 avenue J.Poulhès, TSA 50032
Toulouse 31059
France
Telephone: 33561323200
Fax: 33561323989
Email: kamar.n@chu-toulouse.fr

Saleem Kamili, Ph.D.
Team Leader
Division of Viral Hepatitis
Centers for Disease Control and Prevention
1600 Clifton Road, N.E.
Atlanta, GA 30333
Telephone: (404) 639-4431
Fax: (404) 639-1563
Email: skamili@cdc.gov

Gerardo Kaplan, Ph.D.
Senior Investigator
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
Building 29
8800 Rockville Pike
Rockville, MD 20852
Telephone: (301) 496-0338
Email: gk@helix.nih.gov

Wikrom Karnsakul, M.D.
Assistant Professor
Department of Pediatrics
The Johns Hopkins University School of Medicine
600 N. Wolfe Street
Baltimore, MD 21287
Telephone: (410) 502-9522
Fax: (410) 955-1464
Email: wkarnsa1@jhmi.edu

Yogesh Karpe, Ph.D.
Postdoctoral Associate
Department of Biomedical Sciences and Pathobiology
College of Veterinary Medicine
Virginia Polytechnic Institute and State University
1981 Kraft Drive
Blacksburg, VA 24061
Telephone: (540) 577-7759
Email: ykarpe@vt.edu

Julie Kase, Ph.D.
Microbiologist
Division of Microbiology
U.S. Food and Drug Administration
HFS-711, Room 3E-023
5100 Paint Branch Parkway
College Park, MD 20740
Telephone: (240) 402-2923
Email: julie.kase@fda.hhs.gov

Scott Kenney, Ph.D.
Postdoctoral Associate
Department of Biomedical Science and Pathobiology
Virginia Polytechnic Institute and State University
1981 Kraft Drive, Mailstop 0913
Blacksburg, VA 24061
Telephone: (717) 383-9575
Email: spk151@vt.edu

Jag Khalsa, Ph.D., M.S.
Chief, Medical Consequences Branch
Division of Pharmacotherapies and Medical Consequences of Drug Abuse
National Institute on Drug Abuse
National Institutes of Health
Neuroscience Center, Room 4137
6001 Executive Boulevard, MSC 9551
Bethesda, MD 20892-9551
Telephone: (301) 443-2159
Fax: (301) 443-2599
Email: jag.khalsa@nih.gov

David Kingsley, Ph.D.
Virologist
Agricultural Research Service
U.S. Department of Agriculture
Delaware State University
James W.W. Baker Center
1200 N. Dupont Highway
Dover, DE 19901
Telephone: (302) 857-6406
Fax: (302) 857-6419
Email: david.kingsley@ars.usda.gov

David Kleiner, M.D., Ph.D.
Director, Clinical Operations
Laboratory of Pathology
National Cancer Institute
National Institutes of Health
Building 10, Room 2B50
10 Center Drive, MSC 1500
Bethesda, MD 20892-1500
Telephone: (301) 594-2942
Email: kleinerd@mail.nih.gov

Brittany Kmush, M.S.
Doctoral Candidate
Department of International Health
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street
Baltimore, MD 21205
Telephone: (585) 298-1485
Email: bkmush@jhsph.edu

Kali Kniel, Ph.D.
Associate Professor
Department of Animal and Food Sciences
University of Delaware
044 Townsend Hall
531 S. College Avenue
Newark, DE 19716
Telephone: (302) 831-6513
Email: kniel@udel.edu

Hon-Sum Ko, M.D.
Medical Officer
Department of Dermatology
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
WO22, Room 5336
10903 New Hampshire Avenue
Silver Spring, MD 20903
Telephone: (301) 796-3827
Email: hsko@medic71.org

Seong Ko, M.S.N.
Nurse Practitioner
Hepatology Department
15225 Shady Grove Road
Rockville, MD 20850
Telephone: (301) 987-0020
Email: seko962us@yahoo.com

Christopher Koh, M.D.
Fellow
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 5-4720
10 Center Drive
Bethesda, MD 20892
Telephone: (301) 443-9402
Fax: (301) 402-0600
Email: kohchris@mail.nih.gov

Krishnamurthy Konduru
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
Building 29
8800 Rockville Pike
Bethesda, MD 20892
Telephone: (301) 827-3012
Email: krishnamurthy.konduru@fda.hhs.gov

Rajen Koshy, Ph.D.
Program Officer
Enteric and Hepatic Diseases Branch
National Institute of Allergy and Infectious Diseases
National Institutes of Health
6610 Rockledge Drive, Room 5108, MSC 6604
Bethesda, MD 20892-6604
Telephone: (301) 402-8550
Fax: (301) 402-1456
Email: rkoshy@niaid.nih.gov

Michael Kulka, Ph.D.
Virologist
Division of Molecular Biology
Center for Food Safety and Applied Nutrition
Office of Applied Research and Safety Assessment
U.S. Food and Drug Administration
MOD-1 Building
8301 Muirkirk Road
Laurel, MD 20708
Telephone: (301) 210-7892
Email: michael.kulka@fda.hhs.gov

Alain Labrique, Ph.D., M.H.S., M.S.
Associate Professor
Department of International Health/ Epidemiology
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street, Room E5543
Baltimore, MD 21205
Telephone: (443) 287-4744
Fax: (410) 510-1055
Email: alabriqu@gmail.com

William Lee, M.D.
Professor
Department of Internal Medicine
University of Texas Southwestern Medical Center
POB1.04.420
5959 Harry Hines Boulevard
Dallas, TX 75390-8887
Telephone: (214) 645-6111
Fax: (214) 645-6114
Email: william.lee@utsouthwestern.edu

David Leiby, Ph.D.
Head
Department of Transmissible Diseases
American Red Cross
15601 Crabbs Branch Way
Rockville, MD 20855
Telephone: (240) 314-3541
Email: david.leiby@redcross.org

Vinaya Maddukuri, M.D.
Hepatology and Liver Transplant Fellow
Department of Internal Medicine
Carolinas Medical Center
Annex Building, Third Floor, Room 332
1000 Blythe Boulevard
Charlotte, NC 28203
Telephone: (704) 355-6649
Email: vcmaddukuri@yahoo.com

Sarah Markland, M.S.
Graduate Student
Department of Animal and Food Sciences
University of Delaware
044 Townsend Hall
531 S. College Avenue
Newark, DE 19716
Telephone: (302) 831-6513
Email: sarmark@udel.edu

Participants List

Michael Massare, Ph.D.
Director
Vaccine Development
Novavax, Inc.
9920 Belward Campus Drive
Rockville, MD 20850
Telephone: (240) 268-2000
Fax: (240) 268-2100
Email: mmassare@novavax.com

Mary McLaughlin, B.S.N.
Study Coordinator
Laboratory of Immunoregulation
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 10, Room 8C404
10 Center Drive, MSC 1756
Bethesda, MD 20892-1756
Telephone: (301) 435-8001
Fax: (301) 402-1137
Email: mm149t@nih.gov

X.J. Meng, M.D., Ph.D.
Professor
Department of Biomedical Sciences and Pathobiology
Virginia Polytechnic Institute and State University
1981 Kraft Drive
Blacksburg, VA 24061-0913
Telephone: (540) 231-6912
Email: xjmeng@vt.edu

Ginette Michaud, M.D.
Deputy Director
Office of Blood Research and Review
U.S. Food and Drug Administration
HFM-300
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-6168
Fax: (301) 827-3533
Email: ginette.michaud@fda.hhs.gov

Megan Miller, Ph.D.
Health Science Policy Analyst
Office of Scientific Program and Policy Analysis
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 31, Room 9A05
31 Center Drive, MSC 2560
Bethesda, MD 20892-2560
Telephone: (301) 496-6623
Fax: (301) 480-6741
Email: meganm@mail.nih.gov

Poonam Mishra, M.D.
Medical Officer
U.S. Food and Drug Administration
Building 22, Room 6367
10903 New Hampshire Avenue
Silver Spring, MD 20903
Telephone: (301) 796-1500
Email: poonam.mishra@fda.hhs.gov

Erin Moritz, Ph.D., M.S.
Scientist I
Department of Transmissible Disease
American Red Cross
15601 Crabbs Branch Way
Rockville, MD 20874
Telephone: (240) 314-3535
Fax: (301) 610-4120
Email: erin.moritz@redcross.org

Caryn Morse, M.D., M.P.H.
Staff Clinician
Critical Care Medicine Department
Clinical Center
National Institutes of Health
Building 10, Room 5A06
10 Center Drive, MSC 1403
Bethesda, MD 20892-1403
Telephone: (301) 496-6028
Email: cmorse@cc.nih.gov

Hira Nakhasi, Ph.D.
Chief
Division of Emerging and Transfusion Transmitted Diseases
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
Building 29
8800 Rockville Pike
Bethesda, MD 20892
Telephone: (301) 496-2205
Fax: (301) 480-7982
Email: hira.nakhasi@fda.hhs.gov

Kenrad Nelson, M.D.
Professor
Department of Epidemiology
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street, Room E7132
Baltimore, MD 21205
Telephone: (410) 955-1296
Fax: (410) 955-1836
Email: kenelson@jhsph.edu

Hanh Nguyen, B.S.
Microbiologist
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 50, Room 6525
50 South Drive, MSC 8009
Bethesda, MD 20892-8009
Telephone: (301) 594-2306
Fax: (301) 402-0524
Email: htnguyen@niaid.nih.gov

Hubert Niesters, Ph.D.
Laboratory of Clinical Virology
Department of Medical Microbiology
University Medical Center Groningen
Hanzeplein 1
P.O. Box 30.001
Groningen 9700 RB
The Netherlands
Telephone: 31-50-3615060
Fax: 31-50-3619105
Email: h.g.m.niesters@umcg.nl

Jules O'Rear, Ph.D.
Lead Microbiologist
Office of Antiviral Products
Office of New Drugs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
Building 22, Room 6368
10903 New Hampshire Avenue
Silver Spring, MD 20903-0002
Telephone: (301) 796-0778
Email: jules.orear@fda.hhs.gov

Efi Papafragkou, Ph.D.
Visiting Scientist
Laboratory of Molecular Virology
Center for Biologics Evaluation and Research
Office of Applied Research and Safety Assessment
U.S. Food and Drug Administration
Mod 1 Building, HFS-025
8301 Muirkirk Road
Laurel, MD 20708
Telephone: (301) 210-7812
Email: efstathia.papafragkou@fda.hhs.gov

Keyur Patel, M.D.
Assistant Professor
Department of Gastroenterology
Duke University Medical Center
Box 3850
Terrace Level, Room 0311
2400 Pratt Street
Durham, NC 27705
Telephone: (919) 668-7802
Fax: (919) 668-7164
Email: keyur.patel@duke.edu

Nora Pisanic, Ph.D.
Postdoctoral Fellow
Department of Environmental Health Sciences
The Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street, Suite W7033
Baltimore, MD 21205
Telephone: (619) 892-2563
Email: n.pisanic@gmail.com

Participants List

Sharon Pope, M.S.
Microbiologist
Office of Scientific Program and Policy Analysis
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 31, Room 9A05
31 Center Drive, MSC 2560
Bethesda, MD 20892-2560
Telephone: (301) 435-8130
Fax: (301) 480-6741
Email: popes@mail.nih.gov

Robert Purcell, M.D., M.S.
Section Chief
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 50, Room 6523
50 South Drive, MSC 8009
Bethesda, MD 20892-8009
Telephone: (301) 496-5090
Fax: (301) 402-0524
Email: rpurcell@niaid.nih.gov

Beena Puri, Ph.D.
Microbiology Regulatory Scientist
Division of Molecular Biology
Center for Devices and Radiological Health
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
Telephone: (301) 796-6202
Fax: (301) 847-8512
Email: beena.puri@fda.hhs.gov

Nazia Qazi, M.D.
Chief of Hepatology
Gastroenterology/Hepatology Nutrition Division
Veterans Affairs Medical Center
50 Irving Street, N.W.
Washington, DC 20422
Telephone: (347) 683-4944
Fax: (202) 745-8453
Email: drnazia007@yahoo.com

Abdur Razzaque, Ph.D.
Senior Regulatory Scientist
Division of Emerging and Transfusion Transmitted Diseases
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
HFM-394
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-5355
Fax: (301) 827-9767
Email: addur.razzaque@fda.hhs.gov

Elizabeth Read-Connole, Ph.D.
Program Director
Cancer Etiology Branch
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Suite 5000
6130 Executive Boulevard, MSC 7398
Bethesda, MD 20892-7398
Telephone: (301) 496-6085
Email: bconnole@mail.nih.gov

Rajender Reddy, M.D.
Professor of Medicine
Division of Gastroenterology
Department of Medicine
University of Pennsylvania
Two Dulles, HUP
3400 Spruce Street
Philadelphia, PA 19104
Telephone: (215) 662-4311
Fax: (215) 615-1601
Email: rajender.reddy@uphs.upenn.edu

Gary Richards, Ph.D.
Research Microbiologist
Agricultural Research Service
U.S. Department of Agriculture
Delaware State University
James W.W. Baker Center
1200 N. Dupont Highway
Dover, DE 19901
Telephone: (302) 857-6419
Fax: (302) 857-6451
Email: gary.richards@ars.usda.gov

Annelies Riezebos-Brilman, Dr.P.H.
Department of Medical Microbiology
Universitair Medisch Centrum Groningen
Hanzeplein 1
Groningen 9713 GZ
The Netherlands
Telephone: 31-50-3610223
Email: c.b.m.b.jager@umcg.nl

Elenita Rivera, B.S.N.
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
CRC Building 10, Room 4-5722
10 Center Drive, MSC 1614
Bethesda, MD 20892-1614
Telephone: (301) 435-6125
Email: erivera@cc.nih.gov

Yaron Rotman, M.D.
Assistant Clinical Investigator
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 9C434
10 Center Drive, MSC 1800
Bethesda, MD 20892-1800
Telephone: (301) 451-6553
Fax: (301) 402-0491
Email: rotmany@mail.nih.gov

Niharika Samala, M.D.
Clinical Fellow
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
CRC Building 10, Room 4-5722
10 Center Drive
Bethesda, MD 20892
Telephone: (301) 402-5511
Fax: (301) 451-9160
Email: niharika.samala@nih.gov

Brenton Sanford, D.V.M., M.S.
Biomedical and Veterinary Sciences Program
Virginia Polytechnic Institute and State University
1981 Kraft Drive
Blacksburg, VA 24060
Telephone: (540) 231-7124
Email: bsanford@vt.edu

Souvik Sarkar, M.D.
Clinical Fellow
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 4-5722
10 Center Drive, MSC 1614
Bethesda, MD 20892-1614
Telephone: (301) 451-7009
Fax: (301) 451-9160
Email: souvik.sarkar@nih.gov

Rohit Satoskar, M.D.
Assistant Professor of Surgery and Medicine
Transplant Institute
Georgetown University Hospital
3800 Reservoir Road, N.W.
Washington, DC 20007
Telephone: (202) 444-3635
Email: rohit.s.satoskar@gunet.georgetown.edu

Steven Scaglione, M.D.
Transplant Fellow
Department of Gastroenterology and Hepatology
University of Michigan
3912 Taubman
1500 E. Medical Center Drive
Ann Arbor, MI 48109
Telephone: (708) 359-3744
Fax: (734) 936-0241
Email: stevescaglione@gmail.com

Kathleen Schwarz, M.D.
Professor
Department of Pediatrics
The Johns Hopkins University School of Medicine
Brady 320
600 N. Wolfe Street
Baltimore, MD 21287
Telephone: (410) 955-8769
Fax: (410) 955-1464
Email: kschwarz@jhmi.edu

John Senior, M.D.
Associate Director for Science
Office of Surveillance and Epidemiology
Office of Public Engagement
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
Telephone: (301) 796-0537
Email: john.senior@fda.hhs.gov

Jose Serrano, M.D., Ph.D.
Director, Liver Program
Division of Digestive Diseases and Nutrition
National Institute of Diabetes and Digestive and Kidney Diseases
Two Democracy Plaza, Room 657
6707 Democracy Boulevard, MSC 5450
Bethesda, MD 20892-5450
Telephone: (301) 594-8871
Fax: (301) 480-8300
Email: js362q@nih.gov

Xia Ning Shao, M.D.
National Institute of Diagnostics and Vaccine Development
Xiamen University
400 Siming Road
No. 422 Siming South Road
Fujian 361005
Xiamen
China

Mohamed Tarek Shata, Ph.D.
Associate Professor
Division of Digestive Diseases
Department of Internal Medicine
University of Cincinnati
231 Albert B. Sabin Way, MSB 6360
Cincinnati, OH 45267
Telephone: (513) 558-6110
Fax: (513) 558-1744
Email: mohamed.shata@uc.edu

Adrienne Shearer, M.S.
Research Professional
Department of Animal and Food Sciences
University of Delaware
044 Townsend Hall
531 S. College Ave
Newark, DE 19716
Telephone: (302) 831-0115
Email: ashearer@udel.edu

Averell Sherker, M.D.
Scientific Advisor for Viral Hepatitis and Liver Diseases
Liver Diseases Research Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 659
6707 Democracy Boulevard, MSC 5450
Bethesda, MD 20892-5450
Telephone: (301) 451-6207
Fax: (301) 480-8300
Email: averell.sherker@nih.gov

James Shih, Ph.D.
Professor
Xiamen University School of Public Health
National Institute of Diagnostics and Vaccine Development
400 Siming Road
Xiamen 351006
China
Telephone: 011-86-13950104233
Email: xmujwshih@gmail.com

Krystal Shortidge
Student
Department of Animal and Food Sciences
University of Delaware
044 Townsend Hall
531 S. College Avenue
Newark, DE 19716
Telephone: (302) 831-6513
Email: kshort@udel.edu

Erica Silberstein, Ph.D.
Staff Scientist
Division of Emerging and Transfusion Transmitted Diseases
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
Building 29
8800 Rockville Pike, HFM-310
Rockville, MD 20852
Telephone: (301) 827-3661
Email: erica.silberstein@fda.hhs.gov

Maria Sjogren, M.D., M.P.H.
Hepatologist
Department of Medicine
Walter Reed National Military Medical Center
4800 Wisconsin Avenue
Bethesda, MD 20889
Telephone: (301) 295-5035
Email: sjogrenm@aol.com

Whitney Steele, Ph.D., M.P.H.
Scientist
Department of Transmissible Diseases
American Red Cross
15601 Crabbs Branch Way
Rockville, MD 20855
Telephone: (240) 314-3525
Email: whitney.steele@redcross.org

Barbara Styrt, M.D., M.P.H.
Medical Officer
Division of Antiviral Products
U.S. Food and Drug Administration
Building 22
10903 New Hampshire Avenue
Silver Spring, MD 20903
Telephone: (301) 796-1500
Email: barbara.styrt@fda.hhs.gov

Paul Sue, M.D.
Clinical Fellow
Pediatric Infectious Disease Department
The Johns Hopkins University School of Medicine
200 N. Wolfe Street, Room 3155
Baltimore, MD 21287
Telephone: (410) 614-3917
Email: psue@jhmi.edu

Michele Tana, M.D.
Clinical Fellow
Liver Diseases Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 4-5722
10 Center Drive, MSC 1614
Bethesda, MD 20892-1614
Telephone: (301) 451-1273
Email: michele.tana@nih.gov

Chong Teo, M.D., Ph.D.
Division of Viral Hepatitis
Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30333
Telephone: (404) 819-1267
Email: cteo@cdc.gov

Participants List

Norah Terrault, M.D.
Professor of Medicine and Transplant Surgery
Department of Medicine
University of California, San Francisco
513 Parnassus Avenue, Suite S-357
San Francisco, CA 94143-0538
Telephone: (415) 476-2227
Fax: (415) 476-0659
Email: norah.terrault@ucsf.edu

John Ticehurst, M.D.
Assistant Professor
Division of Medical Microbiology
Department of Pathology
The Johns Hopkins University School of Medicine
Meyer B-119, JHH
600 N. Wolfe Street
Baltimore, MD 21287-7093
Telephone: (301) 580-7811
Email: jticehur@jhmi.edu

Laura Tonnetti, Ph.D.
Scientist
Department of Transmissible Diseases
American Red Cross
15601 Crabbs Branch Way
Rockville, MD 20852
Telephone: (240) 314-3534
Email: laura.tonnetti@redcross.org

Ronald Van Den Bussche, Ph.D.
Regents Professor of Zoology and Associate Dean for Research
Department of Zoology
Oklahoma State University
201 Life Sciences, East
Stillwater, OK 74078
Telephone: (405) 744-5663
Email: ron.van_den_bussche@okstate.edu

Maria Virata-Theimer, Ph.D.
Chemist
Department of Hematology
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
29 Lincoln Drive, Room 304
Bethesda, MD 20892
Telephone: (301) 402-4637
Email: marialuisa.virata@fda.hhs.gov

Qing Wang, M.S.
Graduate Student
Department of Animal and Food Sciences
University of Delaware
044 Townsend Hall
531 S. College Avenue
Newark, DE 19716
Telephone: (302) 831-6513
Email: qingwang@udel.edu

Richard Wang, Ph.D.
Supervisor/Molecular Biologist
Department of Transfusion Medicine
Clinical Center
National Institutes of Health
Building 10, Room 1C711
10 Center Drive
Bethesda, MD 20892
Telephone: (301) 496-9461
Email: rwang@cc.nih.gov

John Ward, M.D.
Director
Division of Viral Hepatitis
Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30333
Telephone: (404) 718-8513
Email: jward@cdc.gov

Kat Whitaker, Ph.D.
Virologist
Department of Microbiology
Office of In Vitro Diagnostic Devices
Center for Devices and Radiological Health
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
Telephone: (301) 796-6208
Email: kathleen.whitaker@fda.hhs.gov

Stefan Wiktor, M.D., M.P.H.
Division of Viral Hepatitis
Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30333
Telephone: (404) 639-8940
Email: swiktor@cdc.gov

David Wilde, M.D., Ph.D.
Medical Officer
Office of Clinical Innovation
National Center for Advancing Translational Sciences
National Institutes of Health
One Democracy Plaza, Room 911
6701 Democracy Boulevard, MSC 4874
Bethesda, MD 20892-4874
Telephone: (301) 435-0799
Fax: (301) 480-3661
Email: wilded@mail.nih.gov

Ningshao Xia
Professor, Dean, and Director
National Institute of Diagnostics and Vaccine Development in Infectious Diseases
School of Public Health
Xiamen University
No. 422 Siming South Road
Fujian 361005
China
Telephone: 86-592-212000
Fax: 86-592-2181258
Email: nsxia@xmu.edu.cn

Chenyu Xu, Ph.D.
Visiting Fellow
Infectious Diseases Section
Clinical Center
National Institutes of Health
Building 10, Room 10N240
10 Center Drive, MSC 1184
Bethesda, MD 20892-1184
Telephone: (301) 640-1667
Fax: (301) 402-1360
Email: xuc@cc.nih.gov

Hailing Yan
Biologist
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
Building 29, Room 304
29 Lincoln Drive
Bethesda, MD 20892
Telephone: (301) 496-1421
Email: hailing.yan@fda.hhs.gov

Christine Yu, M.D.
Microbiologist
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
8301 Muirkirk Road
Laurel, MD 20708
Telephone: (301) 796-0643
Fax: (301) 210-7976
Email: christine.yu@fda.hhs.gov

Mei-ying Yu, Ph.D.
Emeritus/Volunteer
Division of Hematology
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
29 Lincoln Drive
Bethesda, MD 20892
Telephone: (301) 402-4634
Fax: (301) 402-2780
Email: mei-ying.yu@fda.hhs.gov

Jun Zhang, M.S.
Professor
National Institute of Diagnostics and Vaccine Development in Infectious Disease
Xiamen University
Xiamen 361005
China
Telephone: 86-5922183111
Fax: 86-5922181258
Email: zhangj@xmu.edu.cn

Pei Zhang, M.D.
Senior Staff Fellow
Department of Hematology
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
29 Lincoln Drive
Bethesda, MD 20892
Telephone: (301) 827-6579
Email: pei.zhang@fda.hhs.gov

Lilin Zhong, M.S.
Biologist
Department of Hematology
U.S. Food and Drug Administration
29 Lincoln Drive
Bethesda, MD 20892
Telephone: (301) 827-0901
Email: lilin.zhong@fda.hhs.gov

Shimian Zou, Ph.D.
Health Scientist Administrator
Transfusion Medicine and Cellular Therapeutics Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Two Rockledge Center, Room 9144
6701 Rockledge Drive
Bethesda, MD 20892
Telephone: (301) 435-0074
E-mail: shimian.zou@nih.gov

Susan Zullo, Ph.D.
Senior Regulatory Scientist
Division of Emerging and Transfusion Transmitted Diseases
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration
HFM-394
1401 Rockville Pike
Rockville, MD 20852
Telephone: (301) 827-5127
Fax: (301) 827-9767
Email: susan.zullo@fda.hhs.gov

Contractor Support:

Denise Hoffman, CMP
The Scientific Consulting Group, Inc.
656 Quince Orchard Road, Suite 210
Gaithersburg, MD 20878
Telephone: (301) 670-4990
Fax: (301) 670-3815
Email: dhoffman@scgcorp.com

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