

HEPATITIS E

The Leading Cause of Acute Viral Hepatitis in the World

The hepatitis E virus is the leading cause of acute (short-lived) viral hepatitis in the world, and occurs primarily in Africa, Central Asia and Mexico. It accounts for half of all acute hepatitis infection outbreaks in children and adults in areas where it is endemic.

Worldwide, hepatitis E virus (HEV) infection is more prevalent than hepatitis A virus infection. Researchers suspect that as many as 20 percent of the world's population has been infected by the hepatitis E virus. The virus is most common in people between the ages of 15 to 40. In young children, HEV infection often has no symptoms.

In adult populations, hepatitis E can wreak havoc. Since the early 1950s, there have been water-borne hepatitis E epidemics reported in New Delhi, India, the former Soviet Union, Nepal, Myanmar (Burma), Algeria, Pakistan, Cote d'Ivoire (Ivory Coast), Borneo and in refugee camps in Sudan and Somalia.

The virus is transmitted through food or water contaminated by the feces of infected people, but unlike the hepatitis A virus, it is not spread through close person-to-person contact.

Like hepatitis A, an HEV infection never develops into a chronic or long-term illness. However, in adults, hepatitis E is more severe than hepatitis A, with death rates reaching between 1 and 2 percent. In contrast, adult death rates from hepatitis A are less than 0.4 percent.

For some reason that eludes medical researchers, between 15 to 25 percent of pregnant women infected with hepatitis E die, according to the National Centers for Disease Control and Prevention (CDC).

Its symptoms are similar to other types of viral hepatitis, including malaise, anorexia, abdominal pain, jaundice and fever. The acute stage of the disease can last less than two weeks. While symptoms are nonexistent in children and mild in most adults, in pregnant women this infection can be deadly.

Like hepatitis A, there is no treatment for an HEV infection. No vaccine has been developed that prevents HEV infection.

Identification of the Hepatitis E Virus

In 1955, a large epidemic of acute hepatitis swept through New Delhi, India, affecting 29,000 people after raw sewage contaminated the city's drinking water. At the time, health officials assumed it was an outbreak of hepatitis A.

In the early 1990s, scientists tested blood samples that had been drawn and then stored from some of the patients stricken during the New Delhi epidemic. They found a new infectious viral agent that they called enteric (relating to the gut or intestine) non-A, non-B hepatitis.

In a retrospective study conducted in the early 1990s, scientists were able to identify the molecular components of this viral agent and it became known as hepatitis E. The "E" was chosen to illustrate its enteric, endemic and epidemic qualities that capture the epidemiology of this virus. "E" also made sense alphabetically, because hepatitis A, B, C and D viruses had already been identified as causing hepatitis (liver inflammation) in humans.

Hepatitis E, like hepatitis C, is a positive-strand RNA (ribonucleic acid) virus that appears to be a unique agent, and has not been conclusively identified as belonging to any known virus family, though it does share characteristics with the Calicivirus family.

The International Committee on the Taxonomy of Viruses placed the hepatitis E virus in a separate family called hepatitis E-like viruses. Analysis of the virus's RNA shows that the virus forms a genetically distinct group that is closer to a rubella virus than to members of the Caliciviridae family.

When it enters a cell, the virus's genetic material is inserted into the infected cell and "progeny" viruses are then produced by the infected cell.

Most replication of the hepatitis E virus ultimately occurs in the liver, and virus particles are present in the bile and feces of the infected person from late incubation of the virus to the first week of illness.

The incubation period of the virus in humans ranges from three to nine weeks. Patients who show symptoms develop typical acute hepatitis symptoms, such as nausea, anorexia, fever, upper abdominal pain, cola-colored urine and jaundice—yellow coloring of the skin and whites of the eyes. However, the disease can also be silent, with no symptoms at all, which is common in children.

In two human volunteer studies, liver enzyme elevations, which occur when the liver is irritated and liver cells are damaged or die, occurred four to five weeks after oral ingestion of the virus and persisted for 20 to 90 days. During an acute infection, there can also be elevated levels of bilirubin (bile pigments) in the blood and urine, and a mild increase in the alkaline phosphatase, a bile duct enzyme.

In the study, virus excretion in human stool occurred about four weeks after ingestion of the virus and persisted for about two weeks.

Only 1 to 2 percent of non-pregnant patients infected with HEV experience serious or fatal liver disease symptoms. The disease usually resolves itself within two weeks.

It is not clear whether infection with this virus confers any life-long immunity against future infections, as is the case with hepatitis A virus infections.

Hepatitis E in Patients with Existing Hepatitis

In the case of hepatitis A—the other viral hepatitis spread by infected feces—there is potentially a worsening or sudden onset of liver disease among people who contract hepatitis A if they already have chronic hepatitis B or C virus infections. However, there has been little research performed to date to determine to what degree HEV infection accelerates or worsens liver damage in people with chronic hepatitis B or C.

In a letter to *The New England Journal of Medicine*, Dr. Eduardo Bruno Martins of the Federal University of Rio de Janeiro in Brazil reported finding coinfection with hepatitis E and C in 11 percent of 50 adult patients with documented fulminant (life-threatening) hepatitis. He suggests hepatitis E may play a role in causing life-threatening hepatitis in patients with chronic hepatitis C virus infections.

However, to date there is no detailed evidence that HEV infection increases the severity of liver disease in adults or children who already have chronic viral hepatitis.

Epidemiology of Hepatitis E

Historically, researchers thought widespread infection of HEV was found only in India, Central Asia, parts of Africa and in Mexico. According to CDC, currently 40 percent of acute viral hepatitis infections in India result from HEV infection. The largest recorded outbreak of hepatitis E occurred in Xinjing, China in 1986-88 with more than 119,000 documented cases.

In India, 70 percent of HEV infections occur in children. A study by the Department of Pediatric Gastroenterology at the S.M.S. Medical College in Jaipur found 70 percent of pediatric HEV infections were caused by contaminated drinking water and 20 percent by contaminated food.

HEV is transmitted primarily through food and water contaminated by feces from HEV-infected people.

According to the CDC, there is little evidence of transmission through person-to-person or close household contact.

They suggest 9.5 percent resulted from household contact, which is inconsistent with CDC's report that this is not a disease transmitted through person-to-person or household contact. Indian researchers reported a few cases of mother-to-child (vertical) transmission if the mother had hepatitis E during the third trimester of pregnancy.

Hepatitis E viruses were also found in breast milk, but there are no known documented cases of breast milk transmitting the virus to infants.

Among blood donors worldwide, CDC has reported that 11 to 25 percent from developing countries test positive for HEV antibodies.

Scientists cannot confirm that infection with HEV confers life-long immunity against re-infection. Therefore, molecular medicine researchers suggest people may be re-infected over the course of their lives, and can continually contribute infected feces to areas without clean drinking water and with substandard waste-water treatment facilities.

Scientists also wonder whether as-yet unidentified strains or variants of hepatitis E viruses can cause re-infections in developing countries.

Hepatitis E Prevalence in the United States

According to CDC, virtually all cases of acute hepatitis E in the United States have occurred among travelers returning from countries where hepatitis E is endemic, such as India. However, tests on healthy blood donors in the United States show that between 1 and 5 percent have HEV antibodies in their blood. Most of those with antibodies are from urban areas.

The high HEV antibody rate has puzzled CDC researchers. Some suggest the high prevalence of antibodies in the absence of disease and symptoms is due to infection with

a viral strain of hepatitis E that doesn't cause disease, according to Dr. Robert H. Purcell, chief of the hepatitis division in the National Institute of Allergy and Infectious Diseases' (NIAID) Laboratory of Infectious Diseases.

He also suggests that animals may be acting as reservoirs of HEV and passing a weakened form of the virus on to humans.

Non-Human Reservoirs of Hepatitis E Viruses

A study led by researchers at the National Institutes of Health (NIH) suggests that hepatitis E viruses are common among wild rats in the United States. Given the unexpected 1 to 5 percent rate of blood donors in the United States with hepatitis E antibodies, researchers are now examining any potential connection between animals and HEV infection in humans.

Scientists analyzed blood samples from 239 rats captured in alleyways in Baltimore, along the Mississippi River levee in New Orleans and in urban and rural areas of Hawaii. Tests revealed that more than 80 percent of the rats had HEV antibodies in their blood.

Yamina Kabrane-Lazizi of NIAID led the study that included collaborators from the National Institute of Neurological Disorders and Stroke, the Johns Hopkins School of Public Health in Baltimore, and the John A. Burns School of Medicine in Honolulu.

NIAID scientists in Bethesda, Md., have identified a strain of HEV in pigs that is very similar to the strain that causes disease in humans. However, there is no evidence that the pig virus causes disease in either humans or pigs.

To explore infections in pigs, Dr. Xiang-Jin Meng, working with Dr. Purcell and Suzanne U. Emerson screened swine blood from swine from the Midwest for the virus.

According to Dr. Meng, "It's important to remember that the virus strain isolated from the swine in this study is distinct from the strains known to cause disease in humans. Still, further studies are needed to determine whether swine hepatitis E virus is species-specific or is circulating in the human population without causing disease. These sub-clinical infections of humans with swine hepatitis E virus might explain the relatively high prevalence of hepatitis E antibodies in healthy individuals in the United States."

If that is the case, Dr. Meng suggests the strong immunologic cross-reactivity of the

swine and human strains suggests that swine hepatitis E virus could prove useful as a vaccine against the human hepatitis E virus. The similarities between the swine and human viruses also suggest that pigs might provide an alternative animal model for studying HEV infection. Currently, scientists must use expensive primate models to study the virus.

Other major animal reservoirs of HEV antibodies include lambs, cattle and chickens. The animal reservoirs may differ between geographic areas.

Testing and Diagnosing Hepatitis E

Diagnosing hepatitis E in people depends on finding hepatitis E-specific antibodies in their blood and hepatitis E RNA in either blood or stool. In addition, when hepatitis E is acute and symptomatic, there can be increases in liver enzymes, including alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT), which indicate liver inflammation or damage.

When infected, the body's immune system first releases IgM (Immune Globulin Class M) antibodies to combat a foreign substance or antigen, in this case the virus. The concentrations of IgM antibodies quickly decline after three to six months of the onset of an HEV infection. As a result, the quantity of IgM antibodies in blood helps doctors diagnose the stage of an infection.

IgG (Immune Globulin Class G) antibodies are also released to fight the virus. This type of antibody, which is the most abundant, can cross the walls of blood vessels to fight viruses. Hepatitis E IgG antibodies have been shown to persist for two to 13 years after the onset of an infection.

To diagnose hepatitis E in a patient, doctors measure the quantity of IgM HEV antibodies or significant increases in IgG antibodies in a patient's blood. There is currently only one test commercially available that detects HEV IgM and IgG antibodies in blood. Doctors are now working to develop an ELISA (enzyme-linked immunosorbent assay) test for HEV IgM so they can diagnose the disease in its early stages.

There are other tests that can detect hepatitis E RNA in blood and stool, however, the sensitivity of these tests is still under review and most are used primarily by research institutions.

Hepatitis E Genotypes

Researchers are studying the molecular structure and replication of the hepatitis E virus and are just now identifying unique strains or genotypes of the hepatitis E virus.

In a recent study of 29 Chinese residents suffering from acute hepatitis E, scientists identified a unique new strain in 40 percent of the infected patients.

However, research is ongoing and scientists expect new genotypes to emerge as they learn more about the virus. They have already identified molecular differences between hepatitis E viruses found in India, compared to those in Southeast Asia, which are both now classified as Burmese genotypes or type 1.

To date, researchers have identified genotypes by their country of origin, the Burmese, Mexican and United States variants, which are also named genotypes 1, 2 and 3.

In the past two years, a fourth genotype, referred to in some studies as the Chinese genotype, may be responsible for a significant number of cases of acute HEV infection in China.

Why Is Hepatitis E So Dangerous in Pregnant Women?

Hepatitis E is particularly threatening for pregnant women. Fulminant (life-threatening) hepatitis disease can occur, and the death rate for pregnant women ranges from 15 to 25 percent. Hepatitis E is the only hepatitis that apparently has this virulent impact on pregnant women.

Miscarriages or premature delivery can also occur as a result of HEV infection.

Shahid Jameel, in the Dec. 6, 1999, publication of *Expert Reviews in Molecular Medicine* on molecular biology and pathogenesis of hepatitis E virus, suggests the liver's sinusoidal cells can be damaged by the hepatitis E virus, which lessens the ability of these cells to protect hepatocytes against endotoxins that originate from bacteria in the intestinal tract.

He also notes that "hepatocytes can be injured directly by endotoxins or indirectly by eicosanoids, which are 20-carbon chain polyunsaturated fatty acids that cause platelet aggregation, inflammation and other effects. Release of prostaglandins (a type of eicosanoid) can lead to chemotactic attraction of inflammatory neutrophils. This can result in swelling of the tissue by water accumulation (edema) and arrest of bile flow (cholestasis).

“The enhanced sensitivity of pregnant women to such an endotoxin-mediated effect is well recognized and might explain the strikingly high mortality of hepatitis E in pregnancy. However, the validity of this hypothesis and the precise cellular/molecular mechanisms underlying it has not been confirmed,” according to Jameel.

Development of a Hepatitis E Vaccine

Currently, there is no vaccine that prevents hepatitis E. Not even immunoglobulin prepared from plasma collected from hepatitis E infected people is effective in preventing the disease. According to Jameel, studies of experimental hepatitis E vaccines in animals were found to lessen hepatitis E infection, but did not prevent excretion of the virus in the stool of infected, immunized animals.

Two entities, NIH in Bethesda, MD, and Genelabs Technologies in Redwood City, Calif., are now using recombinant DNA technology to create a hepatitis E vaccine. In May 2000, Novavax Inc. and NIH were granted a U.S. patent on an experimental vaccine developed by Drs. Robert Purcell and Suzanne Emerson of NIH and Dr. Robin Robinson, associate director of Novavax's Biomedical Services Division.

The NIH's National Institute for Allergy and Infectious Diseases and Novavax developed a recombinant protein expression system in insect cells for production of recombinant hepatitis E virus capsid antigens and a novel proprietary downstream process system for purifying the viral antigen used as a vaccine.

After successful phase I studies using the recombinant vaccine in 132 volunteers, a phase II efficacy trial was underway in Nepal in mid-2001. A total of 3,000 adult volunteers, ages 18-45 years, are enrolled. The three arms of the study are 5 micrograms, 20 micrograms and placebo. Injections of the experimental vaccine will occur at times zero, 1 and 12 months. The subsequent follow-up will be 24 months, through two epidemic seasons.

The end-point will be the number of cases of acute hepatitis E reported among the three groups.

Future Treatment for Hepatitis E

No treatment currently exists for hepatitis E—the only treatments available address the symptoms, not the disease. No antiviral therapy has been proven to be effective against this virus in controlled laboratory experiments.

Preliminary studies in cell cultures suggest ribavirin and interferon alpha may inhibit hepatitis E virus replication.

According to Jameel, possible drug-target compounds in the virus include some of its enzymes, but extensive research is still required. Other possible targets include blocking interactions of viral and cellular proteins to prevent viral replication.

The only cure is prevention, which will require the purification of drinking water in developing countries and the segregation of wastewater and raw sewage from drinking water sources.

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