

Clinical and Epidemiology of Hepatitis E Virus Infection

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Abstract

The hepatitis E virus, a hepatotropic pathogen transmitted by water and contaminated food, is one of the main etiological agents on the planet of enteral transmission of acute viral hepatitis.

Hepatitis E infections are usually self-limiting, but cases of chronic infection have been described in immunocompromised patients. While self-limiting infections do not require treatment, chronic infections should be treated because of risk of progression to cirrhosis and/or extra-hepatic manifestations.

In Colombia, hepatitis E infections are not included in the routine diagnosis of viral hepatitis, despite evidence of its presence in the country.

The objective of this review is to provide a general description of the hepatitis E virus and the natural history of infections and to highlight studies carried out in Colombia showing its presence in the country. The review was carried out through a search in the PUBMED, SCIELO and ScienceDirect databases for original papers and subject reviews published between 1983 and 2017.

Keywords

Hepatitis E virus, infection, revision, Colombia.

INTRODUCTION

Hepatitis E virus (HEV) was discovered in 1983 when a human volunteer ingested a stool sample from a patient. (1) The volunteer developed a acute viral hepatitis that could not be identified as hepatitis A, B, or C. In the 1990s, the viral sequence was described. (2)

HEV infections are a public health problem, and, according to the World Health Organization (WHO), there are approximately 20 million cases of acute infections every year, mainly in Asia and Africa. (3, 4) The most important transmission route is the fecal-oral route from consumption of contaminated water. HEV is common in areas where the drinking water supply and wastewater treatment are both inadequate. (3, 5) Zoonotic transmission can also occur,

and pigs are the most important reservoir. Transmission occurs as a result of occupational exposure, consumption of poorly cooked pork and water contaminated with fecal material from pigs. (5, 6)

In Colombia, HEV infections have not been recorded because HEV is not included in the viral hepatitis diagnosis guidelines. Nevertheless, there is evidence that the virus circulates in the human population, in the pig population and in the water supply and in sewage. (3, 5-8)

OVERVIEW AND HEV GENOME

HEV is part of the Hepeviridae, Orthohepevirus genus, and Orthohepevirus A species. (9) It is an unwrapped virus of 27 to 34 nm in diameter, but viral particles with lipid

envelopes have also been found circulating in blood. (10) This form allows the virus to evade the humoral immune response. (10, 11) Viral particles released from hepatocytes present a transient lipid bilayer that is slowly lost, first during passage through the bile duct because of the action of deoxycholic acid, and then in the duodenum due to the action of proteases. In the feces, these particles are present in their naked unwrapped form. (12)

The naked viral particle is highly resistant to environmental conditions, so a viral particle isolated from fecal matter can remain stable at temperature conditions below 56° C. However, its infectivity is lost when temperature rises above 60° C, (13) and at 71° C viral particles become inactive in a pig's liver. (14) The HEV particle is resistant to acidic and alkaline pH and to freezing and thawing processes. (6)

The HEV genome consists of a linear single strand of ribonucleic acid (RNA) of approximately 7.2 kb. (6) It has with a positive polarity and contains three open reading frames (ORF): ORF1, ORF2 and ORF3.

ORF1 encodes a polyprotein of approximately 1,690 amino acids and is essential for the replication of the viral genome. (6, 15). The polyprotein is composed of a domain with a methyltransferase (MeT) function, a domain with a protease function, a domain with helicase function (Hel) and a domain with a RNA-dependent polymerase function (RdRp). In addition, it has an X domain and a Y domain with unknown functions (Figure 1). (6) It is not clear whether this polyprotein is processed into individual proteins or if the activity of the domains is conserved in the polyprotein (Table 1). (16)

ORF2 encodes the preORF2 structural subunit whose glycosylated form self-assembles to become a subunit of the viral capsid. (6, 15) This protein has three domains, S, M and P, which are involved in the assembly of the viral particle and in the interaction of the virus with the host cell (Table 1). (6, 16)

ORF3 partially overlaps with ORF2 and encodes a small protein whose function may be to interact with the cytoskeleton for processes of assembling the capsid and the viral particle (Figure 1, Table 1). (6, 18)

Various strains have been identified in patients as well as in domestic and wild animals such as pigs, wild boars, deer, rabbits, mongooses and camels. Importantly, they exhibit

great genetic diversity. (19, 20) Four genotypes characterized in patients have a nucleotide divergence of less than 20% in isolates of the ORF2 region (Table 2). (15)

Table 1. Proteins encoded by HEV and their functions

ORF Genome region	Protein domain	Processes influenced
ORF1	Methyltransferase	Viral replication
	Protease	Viral replication
	Helicase	Viral replication
	RdRp	Viral replication
	X	Unknown function
ORF2	S	Structural subunit of the viral capsid
	M	
	P	
ORF3	-	Interaction with cytoskeleton for formation of capsid, assembly and release of viral particle from cell

Genotype one has been isolated exclusively from human samples obtained during epidemics in Asia and Africa while genotype two has been isolated exclusively from human samples in Mexico and Nigeria. (6, 15, 21) In Latin America, sporadic cases of HEV genotype 1 infections have been reported in Venezuela, Cuba and Uruguay. (22-24) In addition, this genotype has been associated with fulminant hepatitis, abortions and death in pregnant women in countries such as India and Angola. (25) Genotypes three and four have been isolated in sporadic cases of hepatitis in humans, as well as in domestic and wild animals which indicates their zoonotic potential. (15, 20, 21) On the other hand, genotype three has the greatest global distribution and is found in Asia, Europe, Oceania and America while the only documented evidence of genotype four infections is from Asia. (15, 21) Recently, genotype seven has been identified in a report of zoonotic transmission from consumption of meat and camel milk in a liver transplant patient from the United Arab Emirates (Table 2). (20)

Genotype one is subdivided into five subgenotypes which are designated with the letters from a to e. Genotype two has subgenotypes 2a and 2b, genotype three has ten subgenotypes designated with the letters a to j, and geno-

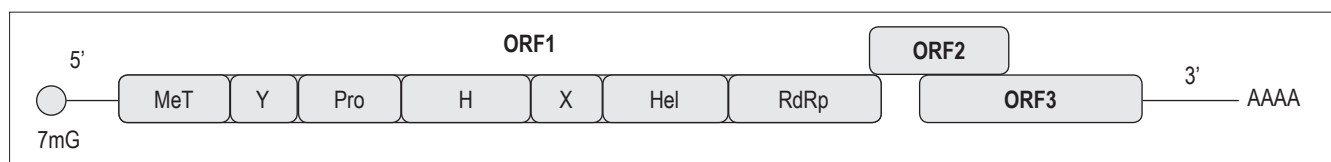


Figure 1. HEV genomic organization. (17) H: domain H; Pro: cysteine-papain protease; X: X domain; Y: Y domain; 7mG: Cap 7-methylguanine. Modified from Panda SK et al. Rev Med Virol. 2007; 17 (3): 151-80.

Table 2. Characteristics and distribution of HEV genotypes that infect humans

Genotype	Sub-genotype	Isolate origin	Transmission	Epidemiological pattern and geographical distribution
1	a-e	Sample of human origin	Contaminated water	Large epidemics in Asia and Africa. Sporadic cases in Venezuela, Cuba and Uruguay
2	a, b	Sample of human origin	Contaminated water	Epidemics in Nigeria and Mexico
3	a-j	Sample of human and animal origin	Contaminated food and exposure to pigs	Sporadic cases in Europe, United States, Mexico, Colombia, Venezuela, Brazil, Uruguay, Argentina, Bolivia and Peru
4	a-g	Sample of human and animal origin	Contaminated food and exposure to pigs	Sporadic cases only in Asia
7	-	Sample of human and animal origin	Contaminated food and exposure to camelids	Sporadic cases in Africa

type four has seven subgenotypes designated with the letters a to g (Table 2). (15)

VIRAL REPLICATION

HEV replication begins with entry of a viral particle into a target cell by endocytosis mediated by a still-to-be-identified receptor (Figure 2). Heparan sulfate proteoglycans

(HSPG) and 70 kilodalton heat shock proteins (HSP70) have been proposed as receptors. (26)

Once the viral particle is in the endosome, the activity of the lysosomal acid lipase (LAL) causes lipid degradation of the particle's membrane. (10)

After entry and decapsulation, the first two-thirds of the viral genome are translated to produce pORF1. (17) Once pORF1 is synthesized, the RdRp domain synthesi-

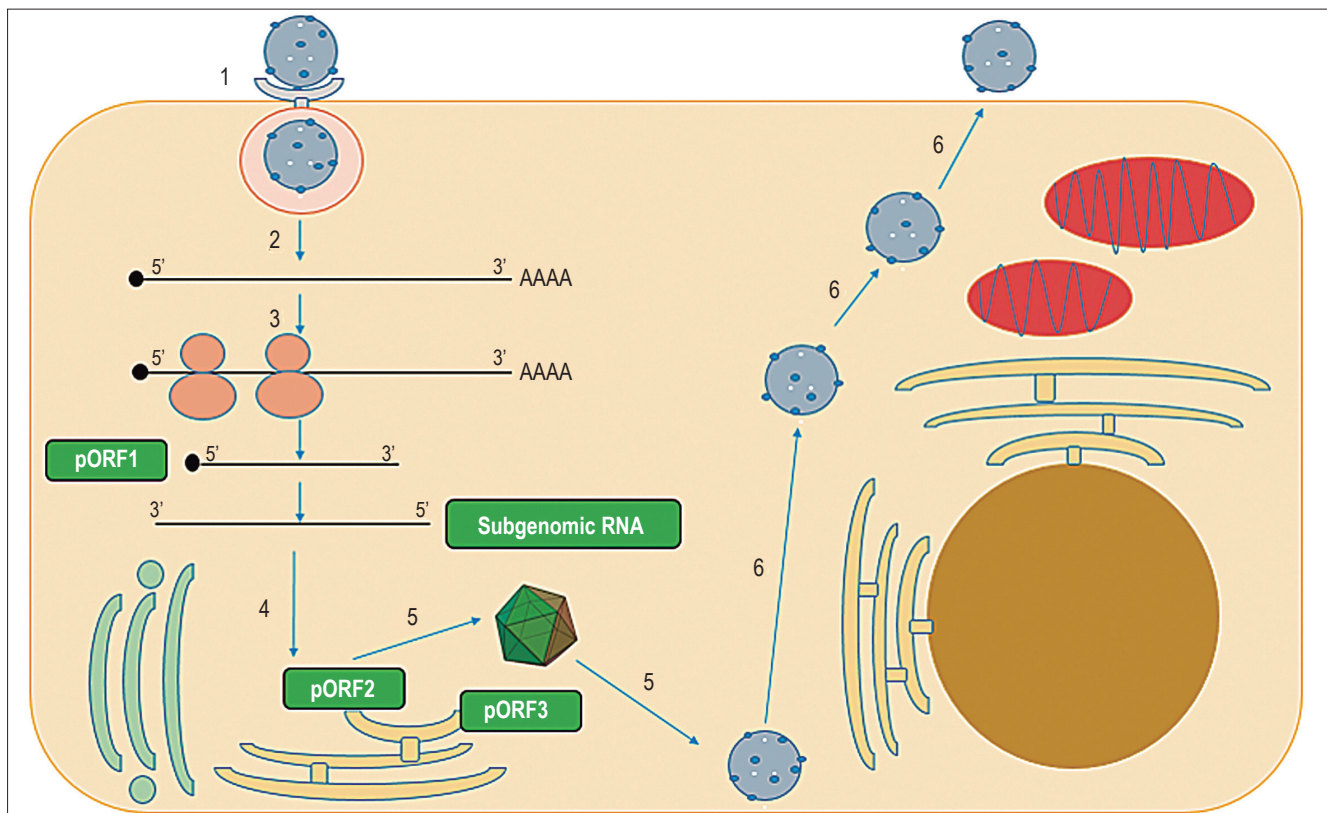


Figure 2. Scheme of HEV replication. 1. Endocytosis occurs with entry of HEV particle into target cell mediated by a receptor 2. Decapsidation and release of RNA 3. translation of nonstructural polyprotein from ORF1 (pORF1) and generation of negative polarity template and subgenomic RNA 4. Translation of pORF2 and pORF3 5. Assembly of the capsid, packaging of the genome and generation of new viral particles 6. Viral particles exit the cell. pORF1: preORF1, pORF2: preORF2, pORF3: preORF3.

zes a complementary polarity RNA chain (antigenomic RNA) that serves as a template for the synthesis of 2.2 kb subgenomic RNA strands and genomic RNA strands. (17) Subsequently, pORF2 and pORF3 proteins are translated from this subgenomic RNA. Then dimers of pORF2 interact which allow self-assembly of the capsid. Subsequently, the genome is packaged and new viral particles are generated (Figure 2). (17, 27)

Although hepatocytes are the main white cells involved in HEV replication, extrahepatic replication has also been demonstrated. Studies in animals have found the HEV genome in organs such as the small intestine, colon, spleen and lymph nodes of pigs and organs such as the kidney, small intestine, spleen and stomach in rats. (28, 29) In addition, genomic and antigenomic RNA has been reported in the central nervous system (CNS) viral replication has been demonstrated in the brain and spinal cord in rodents concomitant with necrosis of neurons, lymphocytic infiltration, perineural invasion and damaged myelin. (30)

EPIDEMIOLOGY

There are 2 epidemiological patterns for HEV infections: epidemic and non-epidemic. (6) The epidemic pattern has been observed mainly in India, China, North and West Africa. In these cases, contaminated bodies of water are the main sources of infection. Usually, the population affected consists of young adults between 15 and 30 years of age. (6, 21, 31) In Latin America, the only outbreaks that have been reported occurred in Mexico in 1986 and 1987. (6, 21)

The non-epidemic pattern occurs in industrialized countries where sporadic cases can be related to the zoonotic character of genotypes three and four. (6, 21)

Prevalence rates are generally higher in developing countries than in developed countries because parts of these populations do not have access to clean drinking water. (6, 21) Areas reported to have high levels of seroprevalence in the general adult population include rural areas of Malaysia (45%), China (20% -30%), Egypt (26%), India (20%) and Saudi Arabia (17%). (6, 21, 32) In developed countries lower seroprevalence rates have been reported in the general adult population. Some examples are rates of the United States (1% -3%), Germany (2.1%) and Spain (2% -7%). (6, 21, 32, 33)

It is important to note that there are differences in seroprevalence of anti-HEV antibodies associated with occupational risk since veterinary care staff and farmers have higher prevalences than does the general population. (34-36) A study in the United States has shown that 27% of veterinarians in eight states with occupational risk have anti-HEV immunoglobulin G (IgG) antibodies which is a much greater percentage than that found in blood donors in the same country. (37) In Moldova, a prevalence of 51%

has been found for anti-HEV IgG antibodies in workers at pig farms compared to a 25% seroprevalence in a control group without occupational exposure to pigs. (36) In Colombia it has been shown that the prevalence of anti-HEV IgG antibodies for pig farm workers varies between 11.25% and 15.7%. (35, 38) In contrast, a prevalence of 2.5% has been reported for anti-HEV immunoglobulin M (IgM) type antibodies in the nearby population. (38) On the other hand, there is serologic evidence that 5.9% of the people who live with pig farms workers in this region have anti-HEV IgG antibodies (Table 3). (38)

Studies conducted in Colombia have reported porcine HEV seroprevalence of 100% for IgG antibodies and 57% for anti-HEV IgM type antibodies. In addition, there is molecular evidence that shows the prevalence of the viral genome in fecal samples from the porcine population ranges between 26% and 41%, and is 60% in liver samples of the same population. (15, 44) Amplified and isolated sequences from the porcine population are genotype 3 subgenotype 3a (Table 3). (15)

Serological and molecular evidence of human HEV infections includes 22.5% presence of the viral genome in stool samples. Amplification has established that this is also genotype 3. (39) Serological evidence shows a prevalence range of 7.5% to 31.2% for anti-HEV IgG antibodies and a range of 1.74% to 11.5% for anti-HEV IgM type antibodies (Table 3). (40, 42)

In addition, 45.2% of the blood samples analyzed from rural blood donors in Yarumal, Antioquia were positive for IgG anti-HEV antibodies (Table 3). (43)

HEV has also been detected in sources of water supply and wastewater in the department of Antioquia. Of 60 samples analyzed, 20% (12/60) were positive for the HEV genome in RT-PCR tests. Genotype 3 was found in water samples from the municipalities of San Pedro de los Milagros, Venecia and Cisneros (Table 3). (8)

CLINICAL PROFILE

In the majority of patients, HEV causes a self-limiting and usually asymptomatic infection. (6, 31) The incubation period lasts for 15 to 60 days, with an average of 40 days. During this time, signs and symptoms develop. These include fever, nausea, abdominal pain, vomiting, malaise and, in some cases, hepatomegaly. (6, 31, 45)

Seventy-five percent of patients with acute infections develop jaundice in the second to fourth weeks after infection (Table 4). (6, 31) HEV can be detected in feces before the onset of symptoms and for up to five weeks later while viral RNA in blood serum is detectable for up to three weeks after the onset of symptoms. (46, 47) Anti-HEV IgM antibodies can be detected during the acute phase of the

Table 3. Studies of HEV infections in Colombia

Study population/samples	Genotype/ subgenotype	Serological evidence	Detection of viral RNA by RT-PCR	Ref.
Patients with a clinical diagnosis of viral hepatitis treated in 5 medical centers in Medellin. Prospective study	3	–	22.5% (9/40 fecal samples) RT-PCR ORF1 and ORF2/3	(39)
Patients with a clinical diagnosis of viral hepatitis from 16 departments of Colombia. Retrospective study	–	11.74% anti-HEV IgM (6/344 samples) 7.5% anti-HEV IgG (26/344 samples)	–	(40)
Workers at pig farms in the Aburrá Valley	–	11.25% anti-HEV IgG	–	(35)
Pigs at slaughterhouses in Antioquia	–	100% anti-HEV IgG 57% anti-HEV IgM	26% (41/152 stool samples) RT-PCR ORF1	(5)
Population with or without occupational risk (pig farms)	–	Occupational risk: 15.7% anti-HEV IgG 2.5% anti-HEV IgM Neighbors of workers exposed to swine due to occupational risk: 5.9% anti-HEV IgG General population: 7.2% anti-HEV IgG 0.81% Anti-HEV IgM	–	(38)
Pig population of farms in the department of Antioquia	3a	–	41.2% (124/300 stool samples) and 59.9% (180/300 liver samples) RT-PCR ORF1 and ORF2	(41)
Patients with HAV, HBV or HCV infections	3a	31.2% IgG anti-HEV 11.5% anti-HEV IgM Coinfection markers: 49% anti-HEV-IgM anti-HAV 31% anti-HEV-HBsAg anti-HBV 41% anti-HEV-RIBA anti-HCV	37% (94/255 serum samples) RT-PCR ORF1 (MeT and RdRp) ORF2	(42)
Water supply and wastewater from 9 municipalities of Antioquia. One municipality in each sub-region of Antioquia	3	–	16.6% (5/30 samples of wastewater) and 23.3% (7/30 samples of water supply) RT-PCR ORF2/3	(8)
Blood donors from the municipality of Yarumal, Antioquia	–	45.2% anti-HEV IgG 0% Anti-HEV IgM	–	(43)

HBsAg: hepatitis B surface antigen; RIBA: Recombinant ImmunoBlot Assay; RT-PCR: Reverse transcription polymerase chain reaction; HAV: hepatitis A virus; HBV: hepatitis B virus; HCV: hepatitis C virus

disease from the fourth day after the onset of jaundice and for up to 5 months after infection. (48) Anti-HEV IgG antibodies may appear simultaneously with the IgM antibody response, but this response increases throughout the acute phase and remains for years after infection. Nevertheless, the exact duration of IgG antibodies is unknown. (48) The appearance of anti-HEV antibodies in serum coincides with the period in which serum transaminases are elevated (Figure 3). (46)

Chronic infections have been associated with genotype three in patients receiving immunosuppressive therapy

for organ transplantation, patients infected with HIV, and patients undergoing chemotherapy (Table 4). (50-52) It should be borne in mind that the epidemiological weight is still unknown, but it is suggested that patients receiving immunosuppressive therapy for organ transplantation and HEV infections may rapidly progress to hepatic fibrosis and then to cirrhosis. (52, 53)

It has been proposed that immunosuppressive therapy be reduced in the first line of treatment since many immunosuppressants including cyclosporine A, tacrolimus and everolimus favor replication of HEV while mycophenolic acid

Table 4. Acute and chronic HEV infections

	Causative genotype	Patient characteristics	Characteristics of infection	Extrahepatic manifestations
Acute infections	1, 2, 3, 4	Patients between 15 and 30 years have infections caused by genotypes 1 and 2. Patients older than 30 years usually have infections caused by genotypes 3 and 4.	Symptoms include fever, nausea, abdominal pain, vomiting and jaundice. Anti-HEV IgM between the second and fourth week after infection.	Neurological disorders: Guillain Barre syndrome Bell's palsy Neuralgic amyotrophy Acute transverse myelitis Encephalitis Hematological disorders: Thrombocytopenia Aplastic anemia Pancreatitis Kidney injuries: Glomerulonephritis
Chronic infections	3	Patients with immune system deficits due to: Immunosuppression following transplant HIV infection Chemotherapy Leukemia	Fatigue is primary symptom. High levels of liver enzymes. Infection can progress to cirrhosis. High concentration of anti-HEV IgG antibodies.	Neurological disorders: Guillain Barre syndrome Bell's palsy Neuralgic amyotrophy Acute transverse myelitis Encephalitis Kidney damage: Glomerulonephritis

HIV: human immunodeficiency virus.

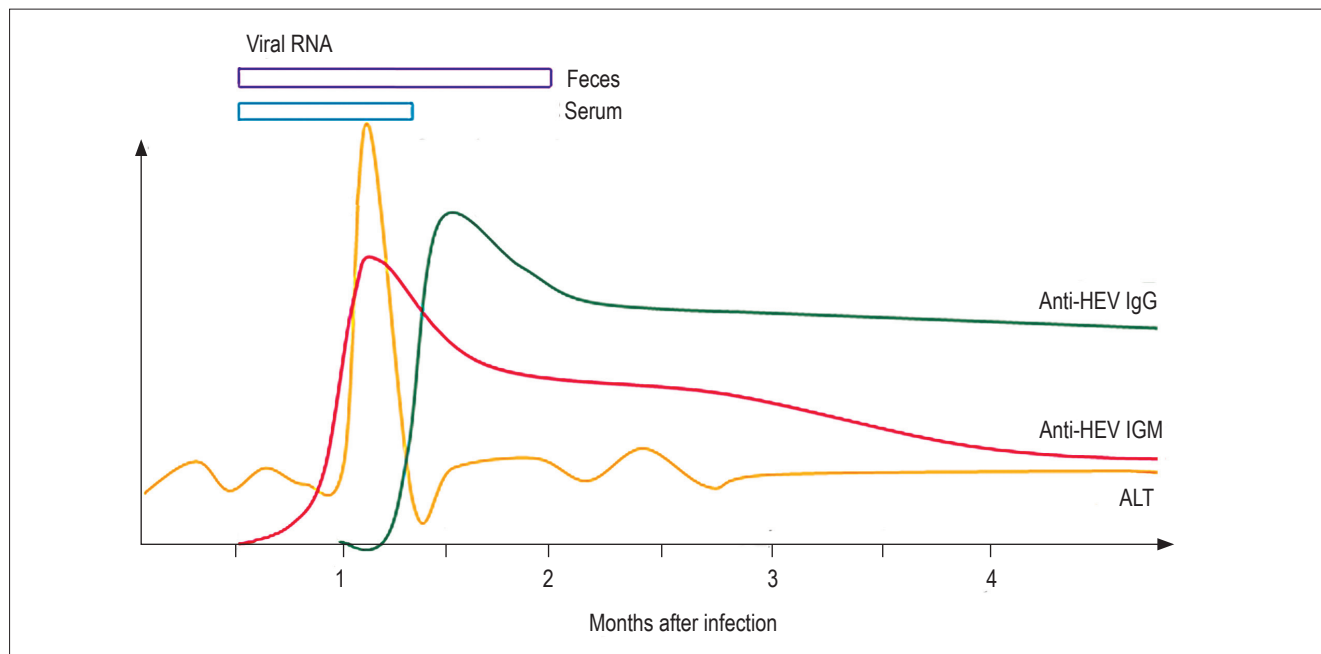


Figure 3. Serological and molecular markers of HEV infections. (49) ALT: alanine aminotransferase. Modified from Aggarwal R. *Semin Liver Dis.* 2013; 33 (1): 30-40.

blocks antiviral activity. (53, 54) If a patient cannot resolve an infection with this strategy, antiviral treatment with ribavirin for three months has had good results. Nevertheless,

antiviral resistance may be associated with mutations such as G1634R. (53, 55) Therapy with pegylated interferon type I has been proposed and has demonstrated moderate in vitro

antiviral activity against HEV. (53) Finally, the drug sofosbuvir has been shown to inhibit virus replication, and antiviral activity increases when it is combined with ribavirin. (56)

Since hepatitis E is clinically indistinguishable from other types of viral hepatitis, diagnosis requires serological and molecular tests. (6) Serological tests are based on detection of anti-HEV antibodies type IgG and IgM by immunoassays which use recombinant proteins or HEV peptides corresponding to epitopes of pORF2 as targets. (6, 47, 48) It is noteworthy that some studies have shown discordant results in sensitivity, and some have even failed to detect IgM antibodies in patients infected with HEV, so false negative results have been generated. Differences between available serological tests could be explained by genotypic diversity, by the antigens used since the antigens used in commercial kits only come from genotypes one and three, or by the methodology used. (47, 49) Since available serological tests vary in sensitivity and specificity, interpretation of results is complicated. (47, 49) For this reason it is recommended that diagnosis of HEV use both serological and molecular techniques to ensure that there are no false negative results. (47)

EXTRAHEPATIC MANIFESTATIONS

Recently, HEV has been associated with extrahepatic manifestations such as neurological and hematological disorders, renal lesions and pancreatitis (Table 4).

Neurological manifestations observed in patients with acute and chronic infection caused by HEV include Guillain-Barré syndrome which is an autoimmune disorder that mainly affects myelin, (57, 58) Bell's palsy which results from damage or trauma of the facial nerves and which causes facial paralysis, (59) neuralgic amyotrophy and bilateral brachial neuritis that mainly affect the shoulders, (57, 60) and acute transverse myelitis which is caused by an inflammatory process in the medulla oblongata. (61)

A retrospective study that included 126 patients with acute and chronic HEV infections found various neurological manifestations in 5.5% (7/126) of the patients. They included Guillain-Barré syndrome, bilateral brachial neuritis and encephalitis. (62) Three of the cases were patients with acute HEV infections without any type of immunosuppression, but the remaining four cases were immunocompromised patients with chronic HEV infections. (62) In the same study, viral RNA was found in the cerebrospinal fluid of four patients with chronic HEV infections which suggested replication and possible passage of the blood-brain barrier. (62)

With respect to hematological disorders, thrombocytopenia and aplastic anemia have been documented in cases of acute HEV infections. (63, 64)

Damage to renal functioning has been described in patients with acute or chronic HEV infections, mainly in liver transplants patients and patients who have taken medications that compromise renal functioning to cause diseases such as glomerulonephritis. (65)

Finally, cases of pancreatitis have been reported in patients with acute infections due to HEV genotype one. (66).

CONCLUSION

It is necessary to alert medical personnel about the importance of including HEV in the diagnosis of viral hepatitis in Colombia given the evidence of this virus in patients, blood donors, pig farms workers and neighbors, pig populations themselves and in drinking and residual water. Although this infection is self-limiting in most cases, it can also progress to chronic infections and to cirrhosis. In addition, it is important to describe the epidemiology of an infection that is emerging in any population in order to control the virus.

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Conflict of interests

The authors declare that they have no conflicts of interest.

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