Hepatitis E virus infection

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Abbreviations:

ALF- Acute liver failure
CLF- Chronic liver failure
CNS- Central nervous system
DNA- Deoxyribonucleic acid
EASL- European association for the study of liver disease
eGFR- Estimated Glomerular Filtration Rate
ELISA- Enzyme linked immunosorbent assay
GBS- Guillain-Barré Syndrome
GTP- Guanosine-5'-triphosphate
HAV- Hepatitis A virus
HCV- Hepatitis C virus
HEV- Hepatitis E virus
HIV- Human immunodeficiency virus
HSM- Hepatosplenomegaly
IgG- Immunoglobulin G
IgM- Immunoglobulin M
LFT- Liver function tests
NA- Neuralgic amyotrophy
NAT- Nucleic Acid Testing
NHS- National Health Services
OCP- Oral contraceptives
ORF- Open reading frame
PCR- Polymerase chain reaction
PNS- peripheral nervous system
Peg interferon- Pegylated interferon alpha
RNA- Ribonucleic acid
SVR- Sustained viral response
Tx- Treatment
UK- United kingdom
USA- United states of America
WT- Wantai Hepatitis E Virus Diagnostic Kits

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1. Summary

Hepatitis E virus (HEV), once thought to occur solely as an acute viral hepatitis in developing countries has gained much better understanding during the past decade. The variety of clinical presentations, ways of transmission and geographic distribution of the several HEV genotypes is much clearer these days, unveiling HEV's true burden.

As of today, potentially attributed to its zoonotic nature, HEV is considered endemic in many developed countries, as well as being the most common cause of acute viral hepatitis in some European countries. There are many more cases of cirrhosis and liver failure, once of unknown etiology, which are now attributed to chronic HEV infection in several patient settings, mostly with deficient immune system, often necessitating treatment. As the viral global disease burden is bigger than once thought, more focus is now being made on recognition, optimal treatment and prevention measures. This overview will focus on the current knowledge regarding HEV, and will cover the microbiological characteristics, epidemiology, pathogenesis, clinical course, and current practices in treatment and prophylaxis.

2. Sažetak

Hepatitis E virus nekad isključivo vezan za razvoj akutnog hepatitisa u zemljama u razvoju, dobiva na značenju tijekom zadanjeg desetljeća. Sadašnje spoznaje vezane uz varijabilnost kliničkih prezentacija, puteva širenja i geografsku distribuciju, otkrivaju pravi opseg problema HEV-a.

Hepatitis E virus je zoonotska bolest, endemična u mnogim razvijenim zemljama te ujedno jedan od najčešćih uzroka akutnog hepatitisa u nekim zemljama Europe. Nadalje brojni slučajevi ciroze i zatajenja jetre nekad nepoznate etiologije, odraz su kroničnog hepatitis E osobito u pacijenata s narušenim imunološkim sustavom, entiteti su koji zahtijevaju liječenje. Budući da je globalno opterećenje virusom danas veće nego što se ranije smatralo, današnji napori usmjereni su prema prepoznavanju, optimizaciji liječenja i preventivnim mjerama. Ovaj pregledni rad nudi sažet prikaz dosadašnjih saznanja o virusu hepatitisa E s osvrtom na mikrobiološke karekteristike, epidemiologiju, klinički tijek te aktualnu prakasu liječenja i prevencije.

3. Introduction

Hepatitis E virus(HEV), although not getting as much attention as other types of hepatitis viruses, became a big research topic, gaining more understanding regarding its prevalence and clinical implications. More than 25 years have passed since identifying the HEV genome, an event which resulted in understanding the true nature, biology, clinical course and possible treatment options for the virus. In the early 1980's, scientists managed to visualize the viral particles in the stool of a healthy patient that was voluntarily infected with feces of others, suspected to carry a non-A, non-B hepatitis virus. (1) In 1990 the virus was cloned, which allowed a year later the development of the first serologic test for HEV. (2,3) HEV is an RNA virus commonly known for its acute hepatitis cases in endemic countries, with approximately 2 billion people at risk of being infected. (4,5) Globally, an estimated 20 million new infections and 70,000 deaths are attributed to HEV in the endemic areas (genotypes 1 and 2). (6) There are probably more cases which are not documented, due to many asymptomatic/mild/extrahepatic presentations of the virus, mostly associated with other genotypes. This overview will focus on the common knowledge regarding HEV, and will cover the microbiological characteristics, epidemiology, pathogenesis, clinical course, and current practices in treatment and prophylaxis.

4. The viral particle

HEV is a non-enveloped, small, positive sense RNA virus, containing 3 open reading frames (ORFs). These are translated into 3 main proteins:

1. The ORF1 protein, containing the functional domains required for viral replication.
2. The ORF2 protein, which is related to the viral capsid, as well as a key component of antigenicity of HEV.
3. The ORF3 protein, a phosphoprotein involved in viral egress and membrane envelopment.

4.1 ORF 1

ORF 1 is the largest encoding area on the HEV genome and represents around 2/3 of its length. It encodes a nonstructural polyprotein of which the function that has been assigned to it is the viral replicas, essential for viral replication. This is based on sequence homology found between ORF 1 domains, and other viruses' domains which are known to be involved in viral replication. (7)

4.2 ORF 2

ORF 2 is responsible for the viral capsid. As such, it has strong antigenic components which led to extensive research in an effort to use it as a tool for developing serologic testing, as well as vaccines. This resulted in ORF 2 being the most extensively studied protein of HEV. (8,9)

4.3 ORF 3

ORF3, a small phosphoprotein, overlaps partially with ORF1 and ORF2 and seem to modulate cellular activities. Despite the protein being implicated in HEV secretion out of the cell, as well as viral membrane envelopment with the host cell's membrane when secreted into the blood,(10-13) the overall limited data suggests that the clear function and structure of ORF 3, remain to be further determined.

5. Classification

HEV contains only one serotype, and several genotypes which are further subdivided. It is also the only hepatitis virus out of the 5 that has an animal reservoir, and has been shown to be also transmitted zoonotically.

HEV belongs to the Hepeviridae family, which is further divided into:

Orthohepevirus (both mammalian and avian HEV isolates) and Piscihepevirus (cutthroat trout). (Figure.1) (14)

Genotypes 1 and 2 are the ones responsible for endemic HEV, which is prevalent mostly in some parts of Asia and Africa, as well as Mexico, and is transmitted fecal-orally, classically through contaminated water. (6,15,16) These are the strains that are more likely to present with acute transient hepatitis in case of a symptomatic disease.

Genotype 3 comprises human and animal strains and is found is developed countries such as, USA, European countries, UK etc. (17) Genotype 4 also includes both human and animal strains which has been isolated in China, Vietnam, Japan, India, France and Italy. (18,19)

Genotypes 3 and 4 (mostly 3) are the ones most often responsible for HEV cases in developed countries, and are considered to cause the documented autochthonous infections in the developed world, unlike past common assumption that most HEV infections/seropositivity was caused by traveling to endemic area. (17,20,21) The different genotypes prevalence in different geographical regions with their different characteristics may be the reason for the different typical clinical picture.

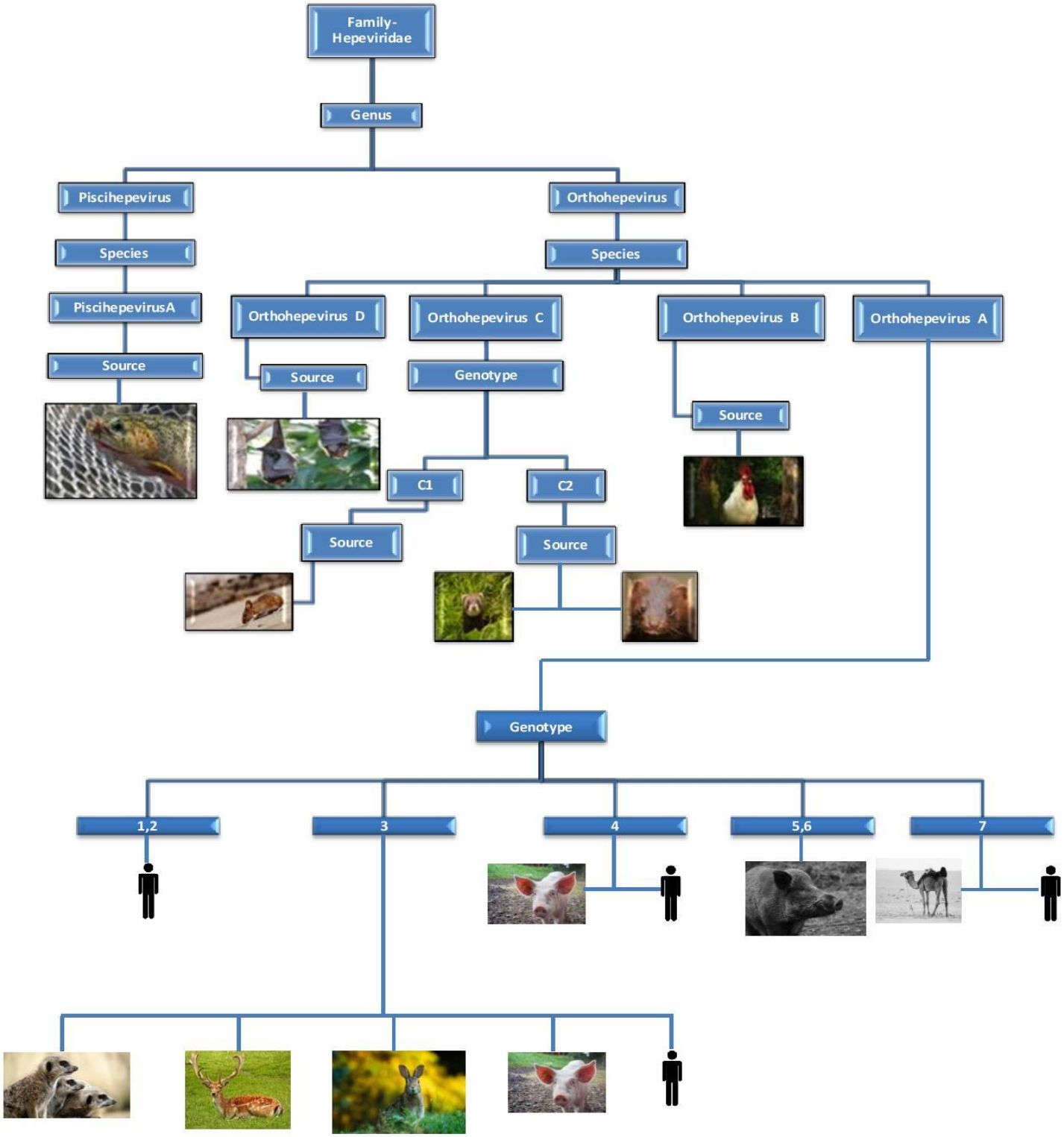

Figure.1: HEV classification and sources, modified according (14)

6. Epidemiology

HEV is classically known to share a "way of transmission" similarity with hepatitis A virus (HAV), through the fecal-oral route. That was well documented in the endemic countries, where large outbreaks of HEV were linked to contaminated drinking water, with genotypes 1 and 2. (15,16) The endemic areas of HEV are mostly Central and Southeast Asia, with documented outbreaks also in the Middle East, North and West Africa, and Central America (Mexico). (22,23) Outbreaks can be either brief or last for more than a year, with the infected population being up to 15%. HEV usually tends to cause an acute self-limited hepatitis when symptomatic. Pregnant women and patients with underlying liver disease however, are considered high-risk groups with the potential for fatal complications. (20) Although there is evidence of person to person transmission of HEV during outbreaks, (24,25) some data suggests that actual person to person transmission is rare, and is more prevalent with family members who shares exposure to the same water source. (26) The trend of HEV way of transmission in the developed countries shifted, as there were more documented cases of autochthonous infections, of which the patient did not travel to an area at risk. (27-29)

Reports also shows that HEV can cross the species barrier, documented by infection of primates (human model) with swine HEV, as well as the infection of lambs, rats and pigs with human HEV. (30-32) Serologic data from pigs, which are considered to be a possible source, showed positive HEV antibodies in number of developed countries, such as USA, Canada, Korea, Taiwan, Australia, and New Zealand. (30,33-35) Another piece of data which supports a zoonotic origin of HEV in developed countries (such as UK, USA, Japan), is high similarity found in phylogenetic analysis and sequencing of many HEV strains isolated from swine and human who shares the same geographical area. (27,30,36) More specific scenarios such as acquiring of HEV through eating raw/undercooked swine liver, (37) as well as wild boar, (36) and deer meat (38) ingestion have also been documented.

The overall seroprevalence of HEV in the developed world is estimated to be 1-5%, which is also found in the healthy population. The main genotype found in developed countries is genotype 3, in contrast to genotypes 1 and 2 in the endemic areas. (29) Genotype 3, besides showing evidence of zoonotic transmission, also shows different clinical course, being mostly asymptomatic, besides specific at-risk groups which will be discussed later. (39,40) The reported seroprevalence is higher than expected, considering the low number of reported infections. The zoonotic nature of HEV

seems to impose a public health concern, especially within people who work with swine. This can prove to be a problem with high magnitude, considering the high consumption of swine products in many of the developed countries. (41-43)

Of note, although not getting a lot of attention is also genotype 7, found in camels as the zoonotic source. Although there's only one documented camel-to-human HEV case to date, of a liver transplant recipient who consumed camel milk and meat, (44) it points to the potential infection source in countries where camel product consumption is prevalent.

Besides the fecal-oral route and zoonosis, transmission of HEV through blood transfusions, (45) as well as vertical transmission, (46) are two other documented routes, which shows increased incidence in recent years.

7. Seroprevalence in Europe

One main focus of HEV seroprevalence in developed countries is the European continent. Immunoglobulin G (IgG) seroprevalence is critical in assessing past as well as present infection in populations. There were several studies being made regarding HEV seroprevalence in Europe, showing a range from 0.6%-52.5%. (47,48) The results, however, are difficult to interpret, as several variables which are taken into account, are different among studies, hence, may influence the results. Those variables that influence the most seem to be the anti-HEV IgG assay, the study group and the geographical region. The majority of the studies show a trend of high HEV seropositivity in Europe, which indicate high exposure rates, which surprisingly comes in contrast to the low incidence of reported acute/symptomatic HEV infections. This finding combined with other data suggests that the vast majority of HEV infections in Europe, like other non-European developed countries, are subclinical and likely unrecognized in many cases. (39,40) It has also been shown that the most dominant type of autochthonous HEV genotype in Europe, genotype 3, tend to follow less severe clinical presentation than the endemic-originated infections, which further supports the presence of under-diagnosing of HEV in Europe due to an asymptomatic course. (49) A big contributor to variability between reported HEV seroprevalence is the type of anti-HEV IgG essay being used. Different assays shows different specificities and sensitivities, a finding which directly influences the assay's performance, and probably the resulting reported data. (50,51) The variability noted in the study group is based on a possible occupational exposure. Higher seropositivity for HEV in people who come in contact with swine and other wild animals

compared to the general population was shown (28% vs 17% respectively) using the Wantai assay (WT). This trend also remained consistent regardless of the type of assay used. (49) These findings are further supported by the fact HEV is highly prevalent in the pig population in Europe. (52) Despite the documented implication of pork product consumption on acquiring genotype 3, (53,54) somewhat surprisingly, France (particularly the southwest part) while having the highest seroprevalence in Europe, has a relatively low pork production relative to other countries in the continent. (55) This raised the assumption that different culinary practices or perhaps different viral loads in certain products (specifically pork) may account for the discrepancy between pork consumption and seroprevalence rates. Many pork products in France contain higher than average viral loads of HEV, one example of which is a pork liver sausage named Figatellu, which is commonly eaten raw, and is connected to several local HEV cases in the southern part of France. (48,56,57) HEV seroprevalence in Europe seem to follow a trend of increasing with advancing age, a finding which is somewhat expected as it may represent a longer chance of exposure. There was no difference found between genders. (55) Geographical region difference is another main variable in different seroprevalence rates in Europe. It seems that the presence of difference between countries is consistent and independent of the type of assay used. France always tops the seroprevalence chart, while Italy always comes last. (Table.1) (55) Besides the noticeable difference between countries, there was also a noted difference within the countries themselves. France is a noticeable example, which while showing the highest reported seroprevalence in Europe, in its southwestern part with 52.5% seropositivity, (48) demonstrates intra-regional variance. (58) UK shows some similar trend, with Scotland measuring the lowest in central Europe (4%), (59) compared to the UK as a whole (13%). (55) This raises the possibility that besides difference in culinary practices, which many times does not vary inside a region's territory, there might be other critical factors, possibly environmental, that influences exposure/seroprevalence. Recent data from 2014-2015 suggests that there is a rise in the reported cases of HEV, even more so than HAV, in a number or European countries such as the UK, Germany, Netherlands and France. (60) Despite that however, there seems to be a decrease in seroprevalence in the UK, Denmark and Germany compared to 20 years ago, (59,61-63) while the Netherlands shows together with increased reported cases also an increase in the seroprevalence of young adults, in conjunction with a rise in HEV viremic blood donors during the course of 4 years. (64-66) There seems to be an overall common trend of increased case-reporting of HEV in Europe, which either

may point to an actual increased exposure risk, or perhaps more likely, better awareness of clinicians to the presence of HEV. There is indeed a documented increase in ordering of HEV testing. (60) Despite the documented increase in reports, the number of documented cases of HEV in Europe may not reflect reality, being much lower than their actual prevalence. This may be related to a lack of proper medical personal education regarding the disease, or possibly due to HEV presenting in a different manner than the expected classic viral hepatitis, such as neurological manifestations, (67) or seemingly iatrogenic liver injury. (68)

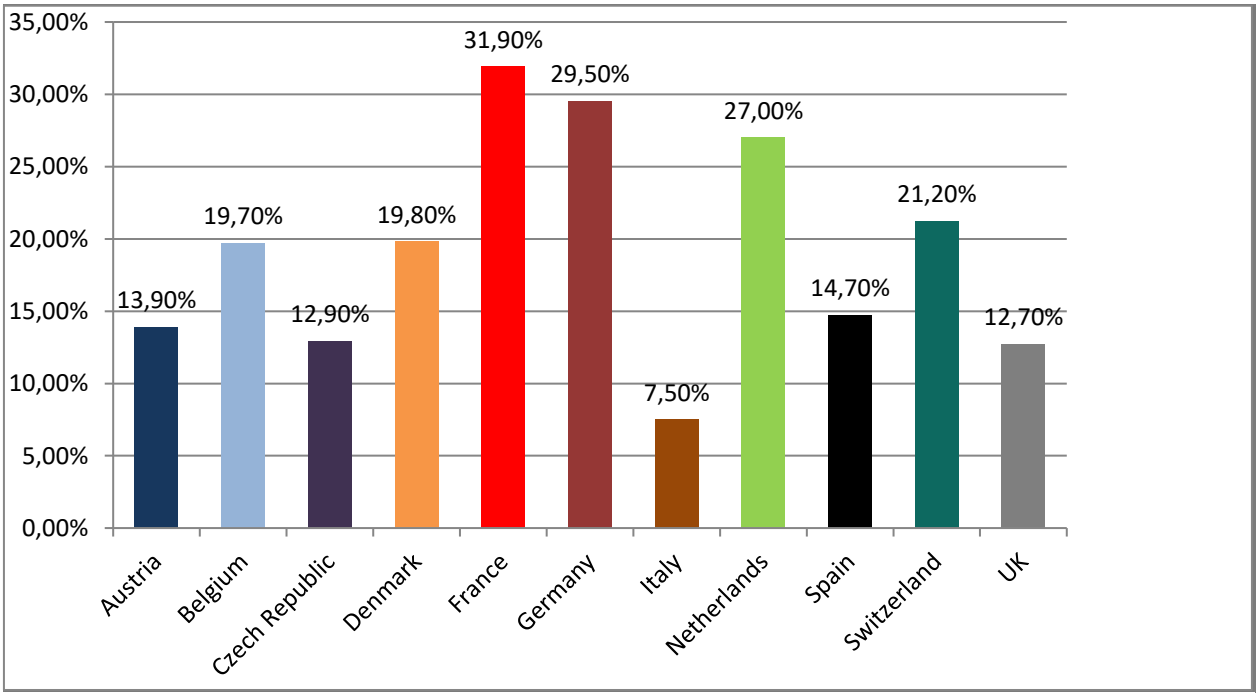

Table.1 Calculated seroprevalence rates for the general population in selected countries in Europe, using the WT assay. Modified according (55).

8. Clinical Presentation

HEV seems to be a bit elusive. While it can manifest classically with typical symptoms of acute hepatitis, similar to HAV infection, it also has other varying clinical presentations, depending on the interaction of the viral genotype, geography, and host factors. This can range from an asymptomatic infection, with or without liver enzyme elevation, all the way to fulminant hepatitis (69) and even purely extrahepatic manifestations.

HEV in its typical symptomatic presentation (usually genotypes 1 and 2 in the endemic regions) has a varying incubation period, ranging from 14 days, to 2 months. HEV typically has 2 distinct period, a prodromal, or pre-icteric phase, followed by the symptomatic icteric phase.

Fecal viral shedding occurs during the pre-icteric phase and diminishing with the initiation of symptoms. (70). The prodromal phase lasts for roughly 3 days and presents mostly with fever, nausea, vomiting, diarrhea, abdominal pain and anorexia. These symptoms typically end with the appearance of jaundice in the icteric phase, which lasts for about 10-14 days. Hepatosplenomegaly (HSM) is common during the icteric phase. (71,72) Asymptomatic liver injury associated with genotypes 1 and 2, although less frequent, is also documented. Fulminant hepatic failure, when it happens, is mostly seen with pregnant women acquiring genotype 1, and patients with pre-existing liver disease. The rates of documented fulminant hepatitis with HEV are higher than HAV. (73) In the non-endemic regions, non-traveling history infection, where genotype 3 is the most common, when the infection is symptomatic, there is predominance in the older male population, usually in the context of preexisting liver disease, alcoholism and immunosuppression. (16,74) The presentation varies, ranging from liver enzyme elevation which is otherwise asymptomatic, to severe acute hepatitis. The most commonly reported symptoms are jaundice, asthenia, fever, musculoskeletal and abdominal symptoms. Extrahepatic manifestations such as neuropathies, hematological abnormalities and pancreatitis were reported as well. (71,72) Despite the still-not-fully characterized interactions of host and viral factors, it seems that different genotypes, host co-infections and the viral load play an important role in determining the clinical presentation. (75) Characterized host factors seem to be: pregnancy and oral contraceptives (OCP) use, (76) pre-existing liver disease, immunosuppression under varying circumstances, (77-80) and age.

Although HEV is mostly known for its acute course when symptomatic, it appears that a chronic course in another possible scenario. All of the documented cases in the developed countries are related to genotype 3. The first case was documented in 2008 in immunocompromised patients,

(74) followed by later documentation of more cases, mostly with a variety of immunocompromised patients spectrum.

9. Vulnerable groups

There has been two main groups in the population which are considered “high-risk” due to the possible increased risk for complications, which divert from the mostly asymptomatic/transient course of HEV.

9.1 Immunocompromised patients - from a wide spectrum of etiologies have been found to be especially susceptible to progression of HEV to a chronic state. This include patients such as solid-organ transplant recipients (77-79,81,82), HIV patients, (83) chemotherapy treatment and hematological malignancies. (71,84) These patients can present in an asymptomatic manner, having only elevated transaminases as the sole evidence of ongoing liver injury. The lack of clinical symptoms can be misleading however, as these patients are susceptible to rapid progression to cirrhosis. (74,78,79) The transmission of the disease in these patients can be either from an infected transplanted organ, (85) as well as eating contaminated meat. Another emerging route of transmission has been through contaminated blood products. (86-88) In addition, a study in Japan found two cases of patients who were tested for HEV serology and RNA both after the transplant, as well as retrospectively in stored serum samples. The pre-transplant serum results were negative for RNA and either weakly positive or negative anti HEV IgG, representing non-exposure and a past cleared infection, respectively. The serum measured strongly positive for anti HEV IgG and RNA post-transplant in both patients, and remained positive, and together with ongoing documented liver injury, was declared as chronic HEV infection. In both cases, the organ donors tested negative for HEV presence, and there was negative history for eating raw meat. Both patients received varying amounts of different blood products, which later showed, when retrospectively tested stored samples, one fresh frozen plasma and one platelet preparation positive for HEV RNA. Sequencing later showed a match in both cases between the transfused blood product and the patient serum viral samples, indicating transfusion related infection. (89) These findings may necessitate stricter approach with blood product screening, especially in the setting of immunocompromised patients receiving transfusion.

9.2 Pregnant women - The associated maternal, as well as fetal and neonatal morbidity and

mortality rates are estimated to be as high as 25%, and have been described with genotype 1, and mostly in parts of India (Noticeably northern India). In contrast, HEV clinical course from other areas such as Europe, USA and Egypt has been reported to be no different than in non-pregnant woman. The prevalence of genotype 1 or some of its subtype in northern India may explain this difference. (90-95) The presentation of an acute infection during the second and third trimesters may be particularly severe; resulting eventually in fulminant hepatitis and liver failure, with high mortality rates (30%-100%). (96,97) Specifically, there is higher rate of maternal cerebral edema and disseminated intravascular coagulation with HEV induced hepatic failure (98). Obstetric and fetal complication that were described include spontaneous abortions, intrauterine fetal demise, post-partum hemorrhage, preterm delivery, low birth weight and premature membrane rapture. (99) Data reported from Bangladesh shows that 58% of the deaths of hospitalized pregnant woman presenting with acute liver disease is associated with HEV infection. (100) It seems there is an interplay between several factors which are modified in pregnancy, such as estrogen and progesterone level which are immunosuppressive, (101-104) may promote viral replication, (105,106) and suppressing hepatic cells, possibly making them more prone to fail if infected. (107) Besides hormones, there are overall changes in the immune function in order to tolerate the growing fetus. This may add an element of immunosuppression and viral favorable environment. Environmental and host genetic factors possibly play a role as well. (93) The relationship is complex, however, it seems to eventually culminate in immune mediated damage as the main mechanism of liver injury. (93) Another piece in the HEV-pregnancy puzzle may be the viral-fetal interaction. Although not fully determined, there is evidence showing HEV replication in the placenta itself (108). In addition, a study done on a rabbit model showed similar findings together with evidence of vertical transmission and adverse outcomes. (109) All of these findings may suggest that fetal infection may play an important role in increased maternal morbidity and mortality.

9.3 Patients with preexisting liver disease - superinfected with HEV are also considered a high-risk group, with increased incidence of fulminant disease and hepatic decompensation. (74,99) This present a complex problem as preexisting liver disease may be compensated, hence unknown to the patient and his physician. This fact, together with the lack of accessibility to HEV vaccine, and

considering the emerging increased prevalence of the disease in developed countries, poses a great challenge for preventive measures in this population.

10. Extrahepatic manifestations

While HEV is classically considered a liver pathogen, There has been emerging cases of extrahepatic manifestations. The most prevalent are myositis, neurological symptoms, hematological abnormalities, and renal abnormalities. (110-114) Interestingly, HEV was shown to replicate outside the liver in animal models. The reports shows that HEV was found in tissues such as the colon, lymph nodes and small intestines of pigs, (115) as well as well as the kidney, stomach and spleen of rabbits. (116) These findings points to possible affinity of HEV to other tissues besides the liver, which may partly explain its extrahepatic manifestations. Neural manifestations which have described in both acute and chronic cases of HEV, include Guillain-Barré syndrome, transverse myelitis, cranial nerve palsies and brachial plexus neuritis (neuralgic amyotrophy). (117,118) A presumed mechanism for the development of such neural manifestations, is believed to be autoimmune related. (119) Impaired renal function with glomerular impairment and cryoglobulinemia has been recognized as an extrahepatic manifestation of HEV, documented in several cases. The association of HEV with renal symptoms in patients, (114,120) who presents with both conditions, is further supported by the presence of HEV in the urine of human patients as well as animals, and one animal even showing immunohistochemically, actual renal cells infection. (121) Other complications which have been described in relation to extrahepatic HEV include hematological abnormalities and acute pancreatitis. (122) These findings suggest the need for increased clinical awareness by physicians of the extrahepatic manifestations of HEV. Another extrahepatic site of possible HEV replication seems to be the placenta, which may be responsible for the severe maternal-fetal HEV associated complications (see vulnerable groups section).

Current European association for the study of liver disease (EASL) recommendations regarding extrahepatic manifestations of HEV includes:

- Patients presenting with neuralgic amyotrophy or Guillain-Barré syndrome

should get tested for HEV regardless of the liver function test (LFT) results. EASL also suggests to test patients which present with encephalitis/myelitis.

- Patients diagnosed with HEV should get tested for proteinuria.

- Renal biopsy is possibly indicated in patients with either acute or chronic HEV with new onset proteinuria.

- Patients with chronic HEV and associated renal disease should get treated with antivirals. (123)

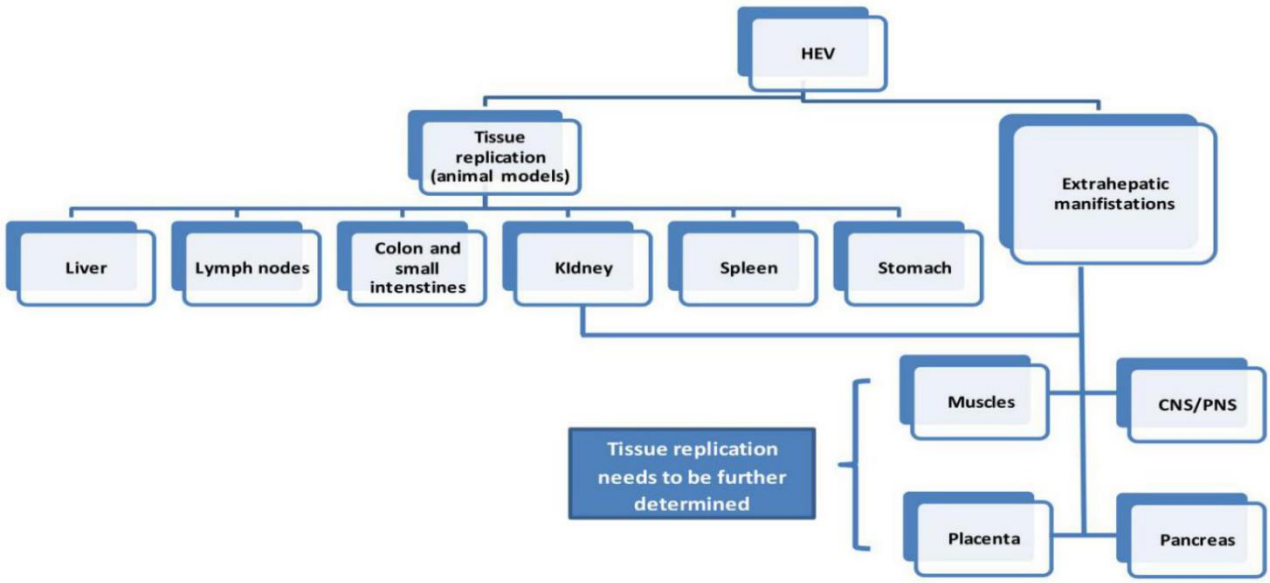

Figure.2 HEV general schematic of confirmed tissue replication in animal models and tissues with documented cases of extrahepatic manifestations. Modified according (124)

Organ/organ system	Clinical manifestation
CNS/PNS	NA
	GBS
	Meningitis/encephalitis
	Mononeuritis multiplex
	Myositis
	Peripheral neuropathy
	Bell's palsy
	Vestibular neuritis
Kidneys	Membranoproliferative and membranous glomerulonephritis
	IgA nephropathy
Hematological	Thrombocytopenia
	Monoclonal immunoglobulin
	Cryoglobulinemia
	Aplastic anaemia
	Haemolytic anaemia
Other	Acute pancreatitis (usually mild)
	Arthritis
	Myocarditis
	Autoimmune thyroiditis

Table.2 Summary of extrahepatic manifestations which has sufficient data to suggest a causal relationship with HEV. Modified according (123)

11. Laboratory work up

HEV diagnosis is generally done either with enzyme linked immunosorbent assay (ELISA) based serologic assays for IgG and IgM antibodies, or with molecular techniques to detect the viral RNA in the serum and feces. There are few available assay kits for detecting HEV specific antibodies, with varying degrees of sensitivities and specificities, in which the results relate mostly to the immunocompetent population. There is still not enough available data regarding the specific performance of the assays in the immunocompromised population, possibly due to decreased antibody response, a finding which raises concern as these patients are specifically susceptible to more complications, requiring a minimal delay in diagnosis and treatment. IgG also proves to be a valuable tool in detecting HEV as it may persist for many years, increasing the chance to detect past/chronic infections.

Another tool besides serology is molecular based testing, using polymerase chain reaction (PCR). The test can be done on blood samples, as well as bile and stool. Since serology can be less sensitive in immunocompromised patients, PCR is an essential tool in diagnosing HEV in these cases. While being potentially seronegative during chronic infection, HEV RNA remains in a detectable range. This value of molecular testing is further strengthened by the fact that other methods of diagnosis such as liver biopsy can be non-specific to HEV, being unable to differentiate it from other common conditions such as other liver associated viruses or drug toxicity in organ transplant recipients. (74) It is important to mention though, that negative PCR does not exclude acute infection. While PCR shows importance in the immunocompromised population, it is somewhat limited in immunocompetent patients, as the period of viral shedding is usually short and transient, occurring only for 1-2 weeks. (71) Regardless, a combination of both methods to diagnose is currently preferred.

Current EASL recommendations regarding diagnostic testing:

- A combination of both serology as well as nucleic acid testing (NAT, eg. PCR) is recommended for HEV diagnosis
- Chronic HEV specifically, due to occurring mostly in immunocompromised patients with possible seronegativity, should also include NAT. (123)

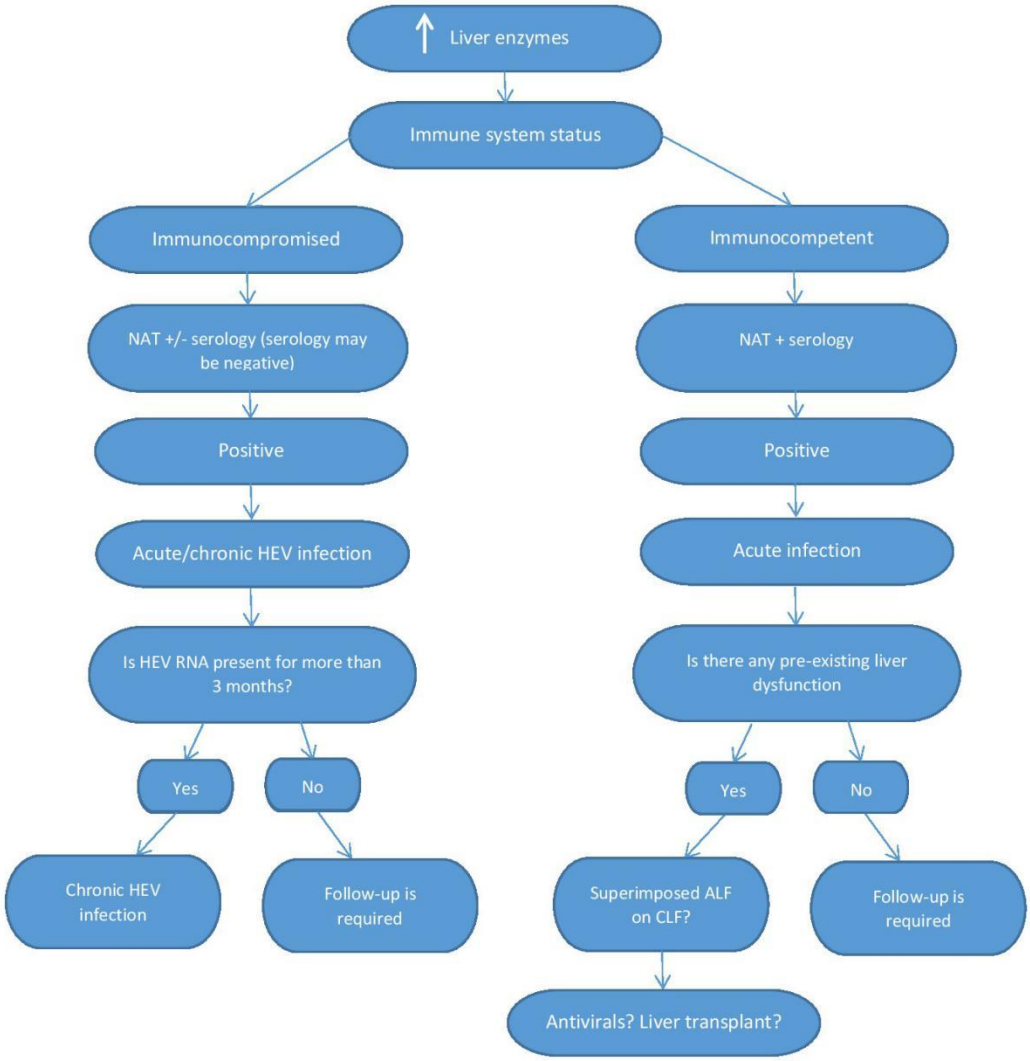

Figure.3 EASL recommended HEV Diagnostic algorithm. modified according (123)

12. Treatment and prophylaxis

HEV represents a therapeutic challenge in several aspects. First, many times the infection is under-diagnosed or missed, something which can cause delays in treatment, possibly affecting the outcome. Second, treatment is still being investigated and is not fully standardized, and third, the patients requiring treatment for HEV usually belong to the high-risk groups such as pregnant, immunocompromised and cirrhotic/preexisting liver disease patients, all of which require special attention when initiating treatment.

HEV when symptomatic, in the vast majority of the cases, causes an acute and self-limiting

disease, requiring nothing but supportive treatment. The main entities of HEV which does require treatment is chronic HEV infection, and fulminant hepatitis with acute liver failure. Liver transplant has been proposed as a possible treatment for cases of fulminant hepatitis with liver failure.

12.1 Acute HEV infection - Despite the fact that acute HEV is cleared spontaneously most of the time, the first line treatment for acute HEV when needed is ribavirin, which showed its efficacy in acute cases. (125,126) Ribavirin has been observed in studies, together with peg-interferon alpha to inhibit viral replication in vitro, as well as having a synergistic effect between the two. A main suggested mechanism proposed seems to be depletion of intracellular guanosine-5'-triphosphate (GTP), although it is still not clear whether there is interplay with other mechanisms as well. (127) Ribavirin, when used, was associated with rapid (within days) viral RNA clearance. Despite documented success with ribavirin, and the achievement of viral clearance in many cases, there are also increasing reports of ribavirin treatment failure with viral resistance. What seems to be a suspected culprit is dose reduction of the drug due to drug induced anemia. (125,128,129). A noted viral mutation responsible for treatment failure is the G1634R mutation in ORF 1 protein. Another point of interest is the fact that although mostly noted in ribavirin treatment for chronic infections, it was demonstrated that ribavirin exerts mutagenic stress on HEV, increasing its heterogeneity and inducing several other mutations besides G1634R in all open reading frames, which were documented in patients with treatment failure.

It is important to note however, that there's also documentation of pre-treatment G1634R mutations on patients treated with ribavirin, which did not impact sustained viral response (SVR) rates.(130) The possible role of HEV RNA variance on treatment efficacy needs to be further explored.

The current EASL guidelines states that in cases of severe acute HEV or a superimposed HEV induced acute liver failure (ALF) in cases of existing chronic liver failure (CLF) ribavirin therapy may be considered. (123)

12.2 Chronic HEV infection - While acute HEV is an entity that usually does not require any therapy, chronic HEV infection, which is defined as being positive for HEV RNA for more than 6 months in the serum/stool, can have devastating consequence, hence, often requiring treatment. It is important to note, that despite the official cut-off for chronicity is 6 months, solid-organ transplant recipients were noted on an observational study to have spontaneous clearance of HEV only in the first 3 months of infection, with no documented cases of clearance between 3-6 months. This data may modify the diagnostic criteria for chronic HEV in the case of solid organ transplant recipients, hence suggesting to initiate treatment for chronic infection after 3 months in that setting. (131)

As up to 60% of immunocompromised patients who has HEV can become chronically infected (77), often when talking about chronic HEV treatment, this is the main targeted group.

A first step approaching chronic HEV in an immunocompromised patient is trying to lower the dose of immunosuppressant drugs, if possible, as it was shown to be an effective on subset of transplant patient. This was based on the observation that lower tacrolimus levels together with daily steroid dosage showed increased viral clearance rates compared to higher levels/dose. (132) The application of this approach has led to almost one third of the patients clearing the infection and developing SVR. (132,133) This approach however is not always possible, making the search for alternative pharmacological methods important. Peg-interferon and ribavirin are essential drugs in chronic HEV treatment.

Both 12 months (134) as well as 3 months duration of peg-interferon therapy (135) showed efficacy in achieving sustained viral RNA clearing. Peg- interferon, however, can increase allogenic immunity, especially in renal transplant recipients, and is generally contraindicated in kidney, pancreas, heart, and lung-transplant recipients.

Currently, according to the EASL guidelines, besides liver transplant patients, peg- interferon is contraindicated in all other solid-organ transplant recipients.

A multicenter retrospective study of 59 solid-organ transplant recipients showed promising results with a 3 months period of ribavirin treatment. 78% of the patients treated showed SVR. In the largest case series, 4 out of 6 kidney transplant recipients achieved sustained viral RNA suppression (136), pointing to potentially prioritizing ribavirin therapy over peg interferon in this patient subset.

While ribavirin does not pose the increased allogenic immunity risk as peg-interferon, data show that ribavirin pharmacokinetics depends on renal function, as well as hemolytic anemia as a potential major side effect.

This side effect usually occurs in the beginning of treatment, and possibly can be managed with a dose reduction. Patient hemoglobin and estimated glomerular filtration rate (eGFR) monitoring is therefore recommended, especially in the first few weeks of initiation of therapy. (137) Other than that, ribavirin generally shows good tolerability by patients.

While currently ribavirin is the first line treatment in chronic infections, despite all the presented data, there is still no placebo-controlled trials to fully support it, as well as to fully define its optimal treatment duration.

Other subset of immunocompromised patients, such as patients with hematological malignancies or HIV can also benefit from peg-interferon and ribavirin treatment, either individually or combined. (138-141) Another option in terms of chronic treatment may be the anti-HCV drug sofosbuvir. Sofosbuvir shows sustained viral response in chronic HCV infections when combined with other drugs (142), and recently has been shown in vitro to inhibit HEV genotype 3 RNA replication. (143) Since sofosbuvir also shows good tolerability in patients, including organ transplant recipients and patients with cirrhosis, (144) it needs to be considered as an optional additional therapy in conjunction with ribavirin, especially when ribavirin monotherapy fails.

Besides lowering or modifying the immunosuppressant's dosages in an effort to clear HEV, the type itself of the immunosuppressive drug itself may have an effect on the viral clearance. Some data shows that the use of mycophenolate mofetil seems to be associated with clearance of HEV. (145) Another example shows that patients that receive tacrolimus has a higher chance of developing HEV chronic infection than patients receiving cyclosporine A. (133)

Current EASL recommendations regarding chronic HEV treatment are:

- Immunosuppressant dose reduction if possible in solid-organ transplant recipient.
- Patients with positive HEV RNA more than 3 months after diagnosis should get treated initially with 12 week course of ribavirin.
- After the treatment interval, HEV RNA should be measured in the serum and stool. If both are negative, treatment cessation is possible.

- If HEV RNA is positive either in the stool/serum after 12 weeks, another 12 weeks course of ribavirin is optional.
- As many solid organ transplant patients have contraindications to peg-interferon alpha, liver transplant recipients who do not respond to ribavirin may be considered for this type of treatment.

(123)

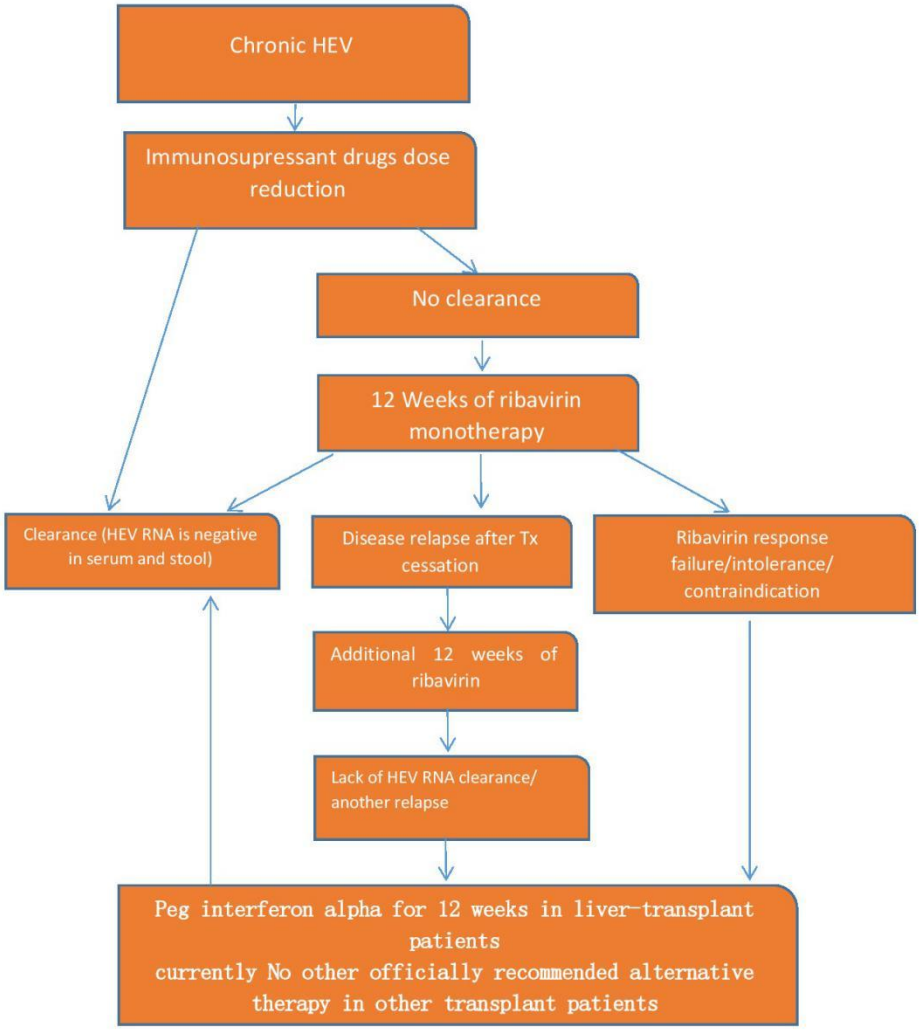

Figure.4 EASL recommended treatment algorithm for chronic HEV infection.
Modified according (123)

12.3 Prevention - As HEV emerges as a global public health concern, together with the potential severe complications and lack of standardized treatment, prevention appears to play a key role in controlling the infection. Several aspects of prevention must be taken into.

In the endemic regions, sanitation plays an important role, where the disease is mostly waterborne. Basic hygiene and prevention of water source contamination is essential.

Particular education and surveillance of vulnerable populations, such as pregnant woman, immunocompromised patients and patients with pre-existing liver disease is also important in order to minimize complications.

The World Health Organization published detailed guidelines regarding waterborne HEV (Figure.5) (146).

In the developed countries, where the zoonotic genotypes 3 and 4 are the dominant ones, special attention needs to be pointed towards increasing the awareness of people who consume raw/undercooked meat, especially from pig, wild boars, deer and rabbit. Even more so, patients with increased risk for developing complications from the mentioned genotypes, such as immunocompromised patients, or patients with preexisting liver disease should be particularly aware of the risks of consuming raw animal products, as well as general contact with these animals.

Proper cooking of food is a critical safety measure to reduce HEV burden in developed countries. Several reports shows that cooking the food thoroughly at 70-71°C, anywhere from 1-20 minutes as well as proper handling and storage of raw pork products can help prevent infection. (147,148)

Swine HEV has been shown to be very contagious between animals, (149) raising the need of people who come in close contact with known HEV carrying animals, such as veterinarians, pig farmers etc., to make sure proper hygiene measures are taken to minimize the risk of transmission. These specific measures are still not fully defined or standardized.

It is probable to say that besides pork which is highly consumed in several parts of Europe, other known contaminated and consumed animal products such as ones from deer and wild boar needs more surveillance. (99) As mentioned above, blood product contamination is also a possible route. In the UK for example, the National Health Services (NHS) Blood and Transplant, recommends that blood products will be routinely screened for HEV in the setting of solid-organ transplant recipients or people undergoing allogenic stem cell transplantation, and be avoided in

case of positivity.
(150,151)

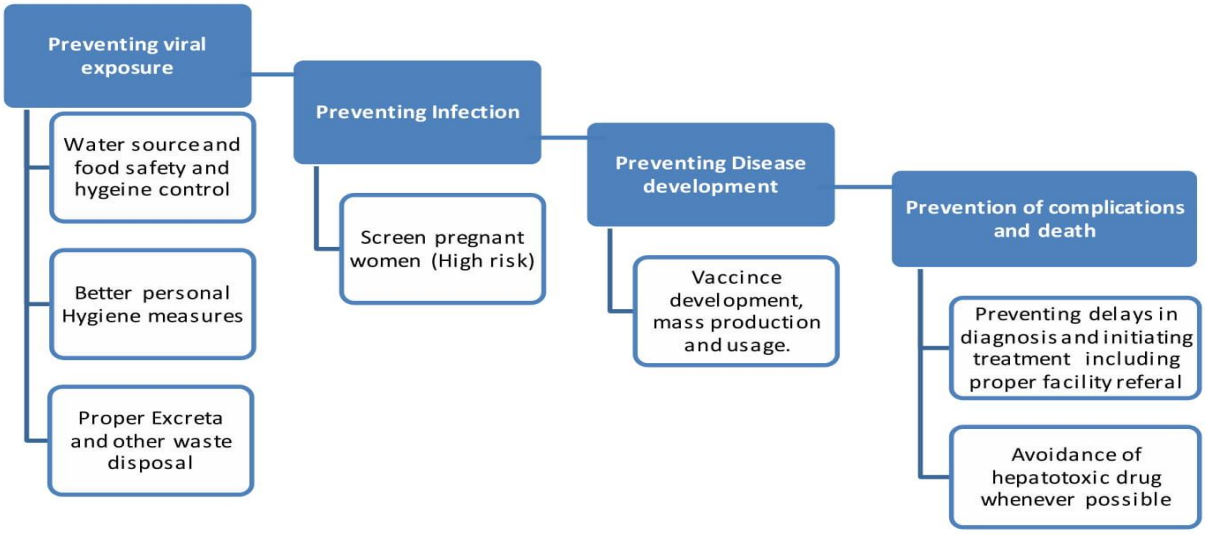

Figure.5 Summary of the WHO's guidelines for controlling waterborne HEV outbreaks.
Modified according (99,146)

12.4 Vaccine - As HEV only has a single serotype, coupled with the fact that acquiring the infection naturally leads to the development of protective antibodies, it seems that HEV is a good subject for vaccine development. (152,153) The first vaccine was based on the highly conserved HEV ORF 2 protein and tested on a sample of 2000 subjects. The vaccine showed an efficacy rate of 95% when all 3 doses were administered, (154) however, despite the promising results, the vaccine was never commercialized.

The second vaccine, Hecolin, was approved by the Chinese State Food and Drug Administration in 2012. (155,156) in trials, it showed 100% seroconversion among subjects who were anti-HEV antibodies negative. (157) The third phase of the trial showed 100% protective efficacy rate among those who received three doses. (158) Furthermore, The long term efficacy of the vaccine, based on a 4.5 year study was determined to be 87%. (159) Despite the vaccine being based on genotype 1, protection against genotype 4 was documented, with genotype 3 protection remained to be further determined. (99) As vulnerable groups in both developed and developing countries are susceptible to the complications of HEV and the fact that currently the only

available vaccine is not commercialized outside of China raises further obstacles in the attempt to minimize HEV's global effects. This suggests that a widely available vaccine seems to be an effective preventive step, which should be highly prioritized.

13. Conclusions

Hepatitis E virus (HEV), an RNA virus, is one of the five known hepatitis viruses. While getting less attention in the past, recent data is starting to show its true magnitude of effect, which seems to be more than once thought. HEV has 2 main presentations, acute and chronic. Genotypes 1 and 2 cause most of the acute infections, transmitted mostly by the fecal oral route, mostly in developing countries. Genotypes 3 and 4 are considered zoonotic, found in many animals and animal products, and cause most of the chronic HEV cases in developed countries. Besides the above mentioned routes of transmission, parenteral and perinatal transmission of the virus seems to emerge as potential routes as well.

Besides the typical liver infection, HEV also can present with a variety of extrahepatic manifestations, neural being the most common one.

While acute HEV infections are usually self-limited and require nothing but supportive care, some groups which are at risk, may require treatment. Pregnant woman in endemic countries seems to be particularly predisposed to complications of acute HEV, while immunosuppressed patients can develop chronic HEV infection with progression to cirrhosis in up to 50% of the patients who acquire the virus. While therapy of HEV is not fully characterized and regulated, some promising findings have been discovered with drugs such as ribavirin, peg-interferon gamma and possibly sofosbuvir, either alone or in combination, being in the center of attention.

HEV appears to emerge as a global public health issue, necessitating an increase in awareness to its existence and clinical manifestations among the medical professionals, and further defining and refining its management and diagnosis protocols.

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16. Biography

Asaf Binderman is a soon to be doctor that studies a 6-year long medical program at the University of Zagreb School of Medicine in Croatia.

He was born and raised in Ramat-Gan, Israel. After finishing his 3-year long army service, about to pursue a career in Veterinary medicine, Asaf decided to follow his true passion and become a medical doctor. Despite having no other family members who work in the medical field, it is something that Asaf always wanted. After spending 6 years in Croatia, passing all the exams and receiving the Deans award in his first year of Medical school, he is ready to move back to Israel in order to pursue his medical career and live there with his wife and daughter. The period spent in Zagreb is something he will always remember positively.