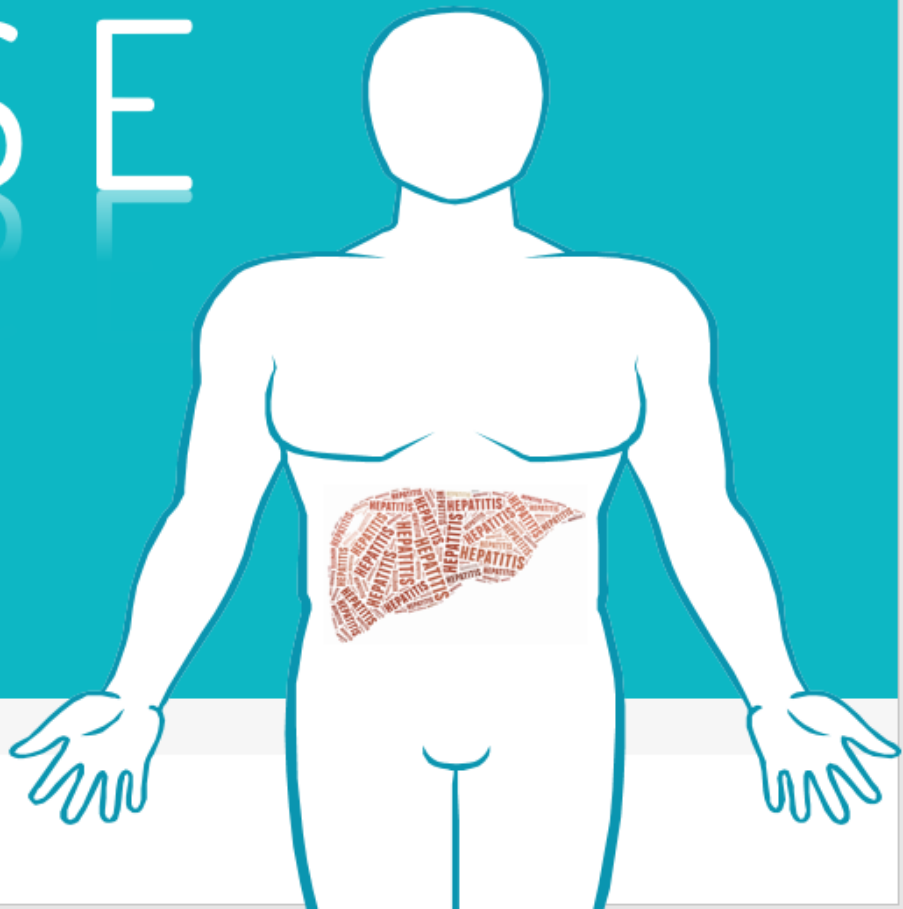
HEPATITIS E

FROM FOOD OF ANIMAL ORIGIN



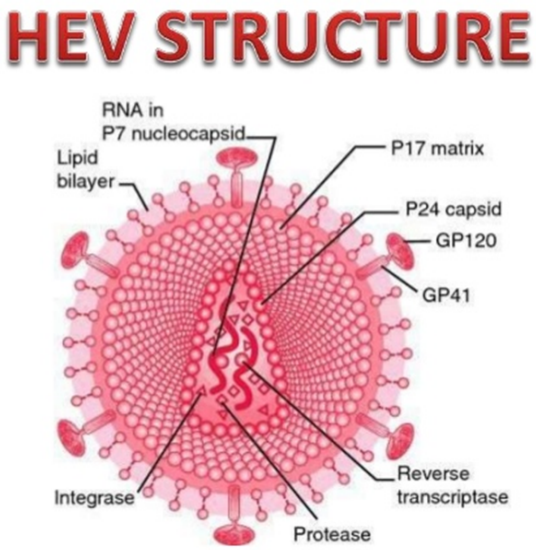
INTRODUCTION

Hepatitis E is a liver disease caused by the Hepatitis E virus (Hev).

It is particularly common in developing countries where lack of water and precarious hygiene conditions promote the spread of the virus.

Hepatitis E virus (HEV) is the major etiologic agent of non-A hepatitis enteric, and is the only member of the Hepevirus class of the Hepeviridae family

The discovery of hepatitis E came in the light of lime when the outbreak of Kashmir hepatitis of 1978 was investigated.

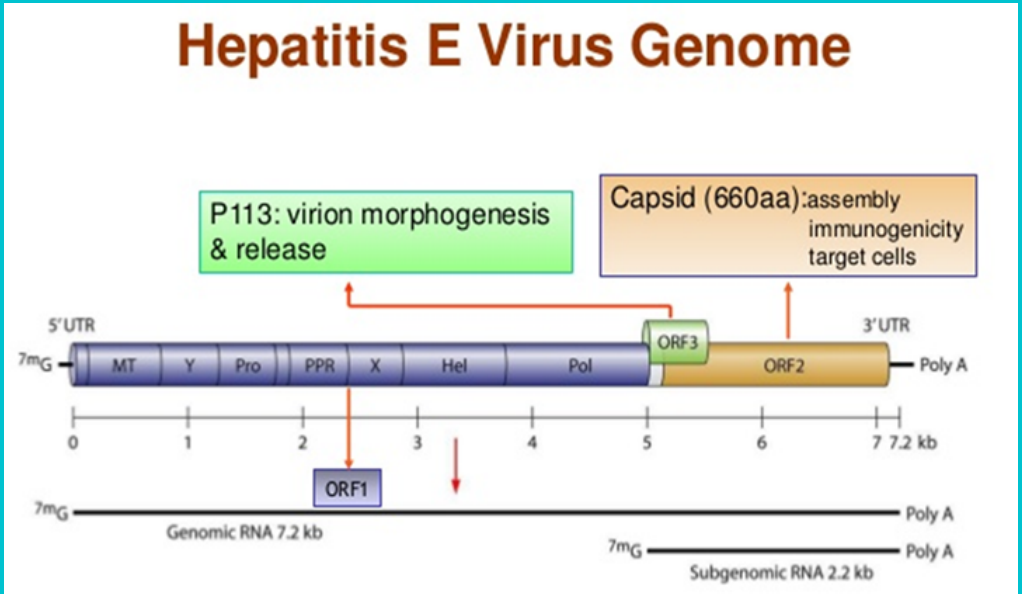


STRUCTURE

HEV was first visualised in 1983 by using immunoelectron microscopy. HEV belongs to the genus Hepevirus, in the Hepeviridae family.

Includes viruses that infect humans, domestic pigs, wild boar, deer, and rodents.

Avian HEV has a genetic structure that is partially different from the others.



Is a positive-sense small [27–34 nm] single-stranded RNA non-enveloped virus with an icosahedral capsid.

The viral genome, of about 7.2 kb, is made up of a short untranslated region of 27-35 nucleotides followed by 3 open reading frames (ORFs) and a second untranslated region of about 65-74 nucleotides, with a poly A sequence at the 3'-terminal polyadenylated end of 39-72 nucleotides

HEV is classified based on the nucleotide sequences of the genome and is now characterised as a single serotype with four major genotypes [HEV 1–4], with each genotype having several subtypes.

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CLASSIFICATION

- Genotype 1 (1a – 1e): has been isolated from both tropical and some subtropical countries in Asia and Africa
- Genotype 2 (2a and 2b): have been isolated in Mexico, Nigeria and Chad
- Genotype 3 (3a – 3j): have been found worldwide – Asia, Europe, Oceania, North and South America
- Genotype 4 (4a – 4g): limited isolation, mainly in Taiwan and China.

Table 1. Hepatitis E virus genotypes.

Characteristics	genotype 1	genotype 2	genotype 3	genotype 4
Geographic Location	Africa and Asia	Mexico, Nigeria and Chad	developed countries worldwide	China and Taiwan
Viral discovery	1983	1986	1995	2003
Subtypes	5	2	10	7
Transmission route	water-borne faecal-oral person to person	water-borne faecal-oral	food-borne	food-borne
Groups at high risk for infection	young adults (15–30)	young adults (15–30)	Adults aged over 50 and male immuno-compromised persons	young adults
Zoonotic transmission	no	no	yes	yes
Chronic Infection	no	no	yes	no
Outbreaks	common, can involve thousands of cases	smaller scale outbreaks	uncommon	uncommon
Transfusion related infection	yes	yes	yes	yes

- Developing countries: genotypes 1 and 2, which are restricted to humans, and is especially associated with males, with larger outbreaks and epidemics in developing countries with poor sanitation
- Industrialised and developing countries: genotypes 3 and 4 which infect humans, pigs and other animals, and have been responsible for sporadic outbreaks

TRASMISSION

HEV is transmitted by the **oral route** and occasionally through the **parenteral route** after accidental transfusion of HEV positive blood donation.

The band age group most affected are between 15 and 44 years.

Cases of human transmission are rare, person-to-person contact, are considered to be too inefficient to represent a significant risk of HEV transmission.

TRASMISSION

Hyperendemic areas

hepatitis E is often linked to the contamination of water resources with human faecal material

Low-endemic areas

high socioeconomic development, hepatitis E is predominantly a food-borne zoonosis

TRASMISSION

Domestic pigs are the main animal reservoirs of HEV worldwide.

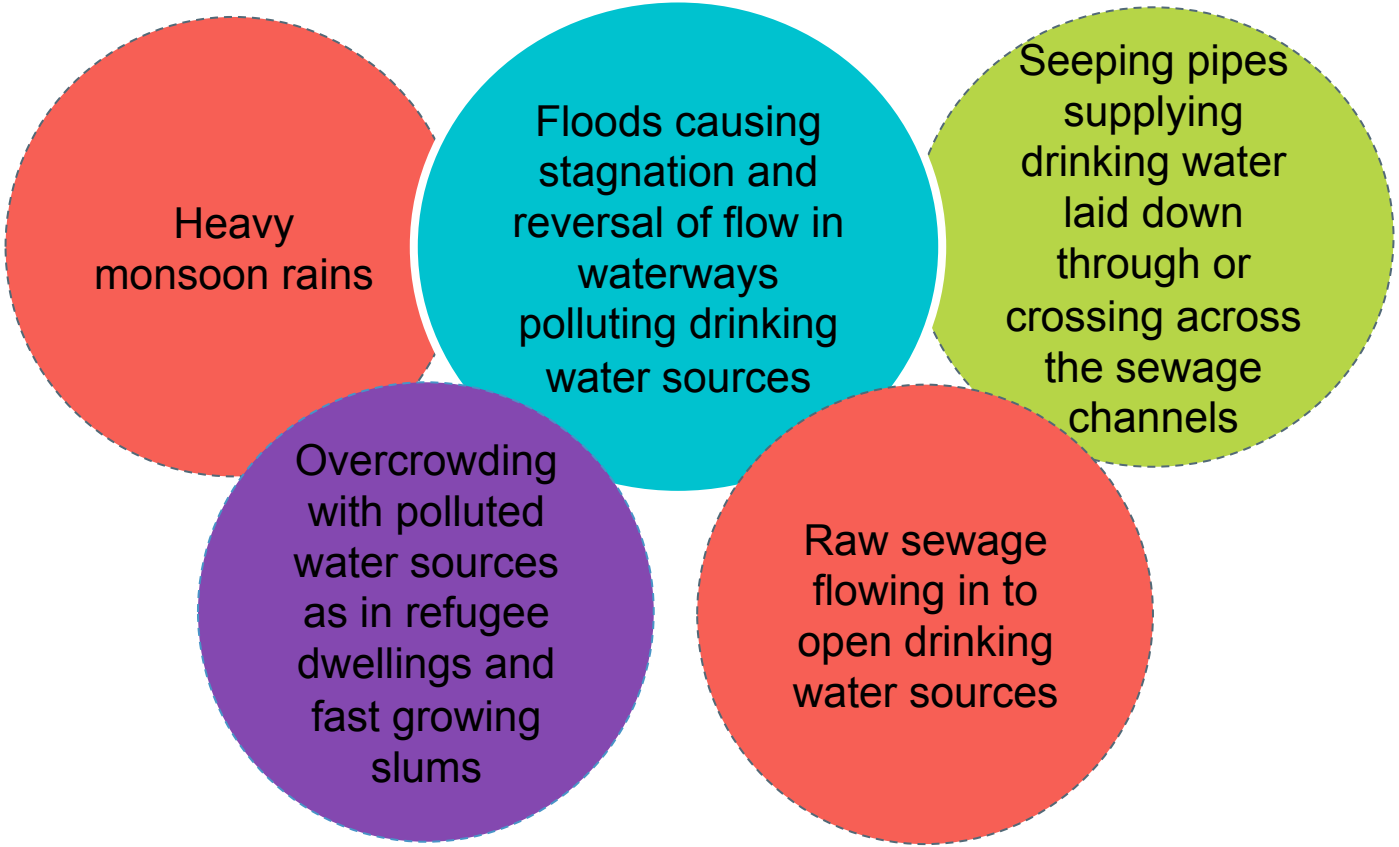
Was also transmitted to humans who consume meat or milk from wild animals, including wild boar, deer, rabbits and camels.

There is a need to review the available methodologies for the **detection**, **characterisation** and **quantification**.

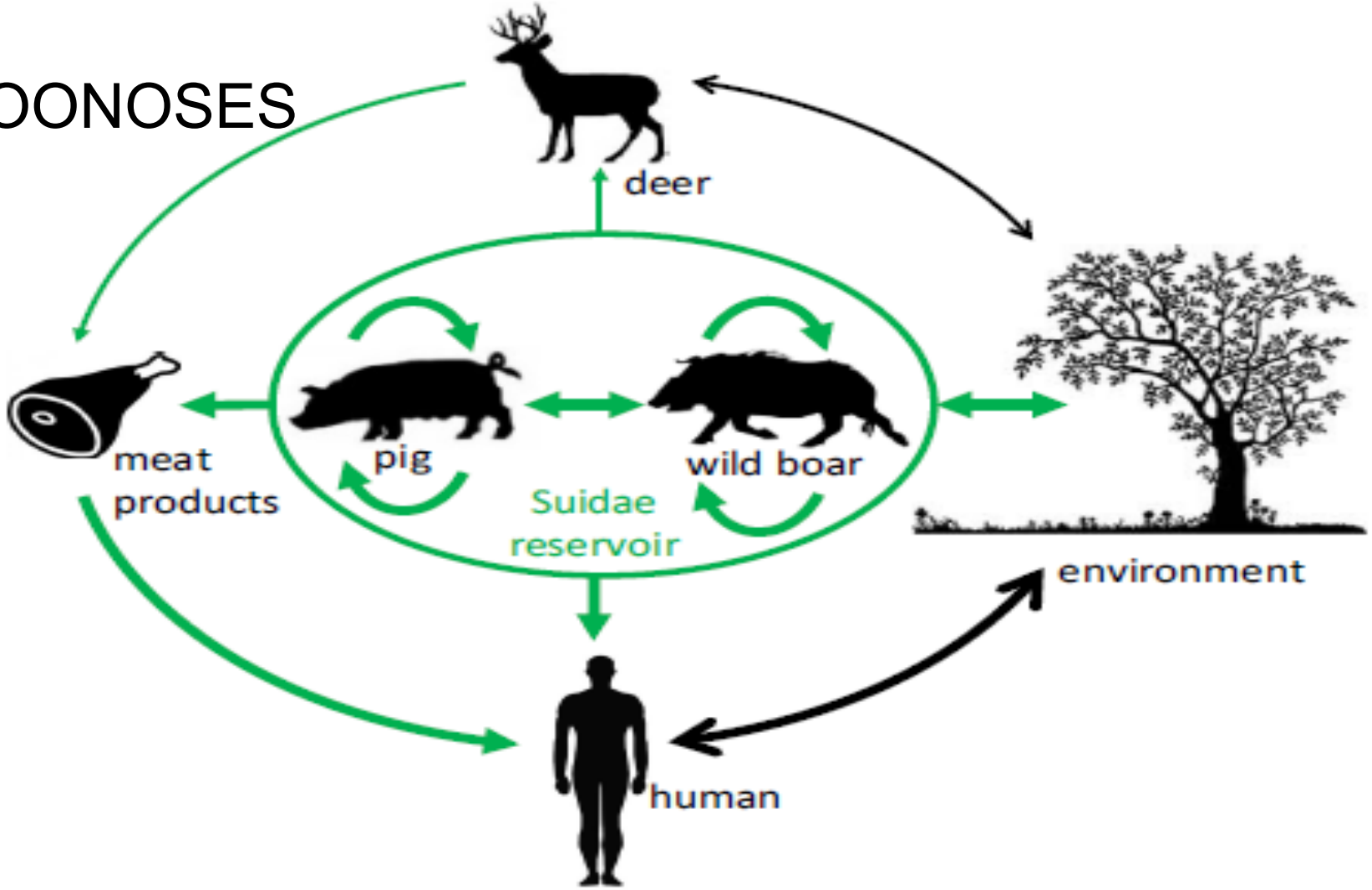
Importance of food as a source of infection.

WATERBONE TRASMISSION

WATERBORNE TRASMISSION



13 ZOONOSES


deer
meat
products
pig
wild boar
Suidae
reservoir
environment
human

HEV is suspected to be mostly a foodborne disease transmitted through consumption of infected food products.

- Food originating from an animal infected with HEV
- Food products contaminated with HEV

HEV is widespread in pig farms and consumption of pork products, specially containing pig liver, is associated with HEV infections

HEV is also present in wild boars

The environmental contamination from human/animal sources may have a role in the dissemination of HEV

The virus has been detected in urban sewage,

Transmissions through direct contact with infected animals

Contact exposure with infected animals is a possible transmission route as well, since professional occupations with animal reservoirs have a higher seroprevalence than the related general population.

There is an urgent need for a realistic evaluation of the burden of HEV disease in humans in general and in specific risk groups

Transmissions through direct contact with infected animals

Contact with infected pigs or their organs was reported as a confirmed source of HE, but living in a pig-dense area where farm biosecurity and waste management is carefully controlled was not associated with increased risk.

Most evidence for this transmission pathway comes from serological studies analysing persons with occupational contact with animals compared to a control group.

PIG

The transmission of this enteric virus between pigs is strongly influenced by environmental faecal contamination, which suggests the possibility to reduce the prevalence of infected pigs by appropriate farm management, hygiene and biosecurity measures.

The virus was also detected in the urine of pigs infected with HEV, making urine a potential route of transmission

Risk factors associated with HEV infection within pig farms

A possible way to reduce human exposure through consumption of infected meat is to reduce the number of infected animals at slaughterhouse.

- early slaughtering of young animal,
- genetic line of the female breeding stock,
- lack of biosecurity measures
- use of drinking water from a nearby source

High seroprevalences at the end of the rearing period were associated with excessive mixtures of post-weaning animals and low conditions of hygiene

WILD FAUNA

Wild boar is an important reservoir of HEV.

Hepatitis E virus has also been detected in **deer**: however, lower HEV seroprevalences and HEV RNA detection rates are frequently found in deer compared to wild boars sampled within the same geographical region

Deer are not a true reservoir of HEV but are infected accidentally by sharing the same habitat as wild boar

HEV transmission between domestic and wild Suidae

Experimental infections have shown that HEV can cross the domestic pig-wild boar barrier

↓

Wild boars naturally infected or inoculated intravenously with HEV are able to transmit the virus by contact to domestic pigs

↓

These data strongly suggest that transmission via the fecal– oral route between domestic and wild swine is possible and can occur naturally when pigs and wild boars are reared in close contact

RABBIT

HEV isolated from rabbits is genetically distinct from strains detected in most human HE cases, and has been found in farmed rabbits in several geographic areas of China, USA and France.

Sequence analysis indicates that all the strains from rabbits belonged to the same clade, which represents a distinct subtype closely related to HEV-3.

HEV occurrence and persistence in food

There is direct (detection of HEV RNA) and epidemiological evidence that supports the link between the onset of HE and the consumption of HEV contaminated food of animal origin, causing both sporadic cases and outbrakes of infection.

HEV occurrence and persistence in food

Domestic pigs and wild boars are the most important reservoirs and also the species most investigated

- Liver and liver product
- Meat
- Milk
- Shellfish
- Blood product
- Food of non animal origin

Stability of HEV in the environment

Hepatitis E virus can persist in the farm environment but survival parameters are mainly unknown.

Experiments performed with HEV in cell culture supernatant indicate that infectious HEV can be detected after one month storage at room temperature and after more than 2 months storage at 4 °C.

The virus is mainly excreted fecally in pigs, leading to an accumulation of HEV in the environment of infected livestock.

Thus, both contact between individuals and environmental exposure can play a role in HEV transmission. Experimentally, a minimum load of 106 copies of HEV RNA/g appears necessary to infect pigs per os and cause excretion of the virus and transmission to their congeners.

DISCOVERY

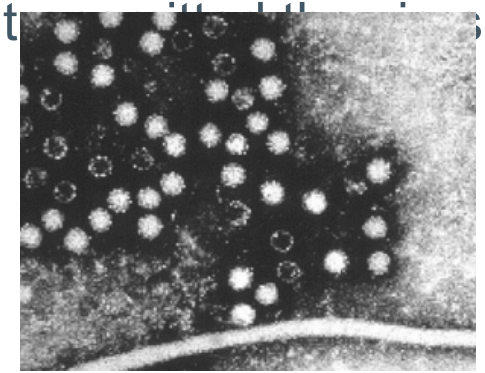
The 1978 epidemic

DISCOVERY

- 1978 non-A, non-B hepatitis (NANBH) Kashmir (India) epidemic:
- 52000 patients with icteric disease and 1700 fatalities
- The disease affected young adults within the age group of 15 to 45 years
- It was particularly severe in pregnant women
- The agent was able to be transmitted vertically with high fetal and perinatal mortality

DISCOVERY

- In 1983, an outbreak of NANBH was reported in Russian military personal posted in Afghanistan
- Dr. Mikhail S. Balayan ingested pooled stool extracts from 9 patients
- After 36 days he developed severe acute hepatitis
- Stool samples showed virus like-particles (VLP) on IEM
- He successfully t[illegible]s to cynomolgus monkeys



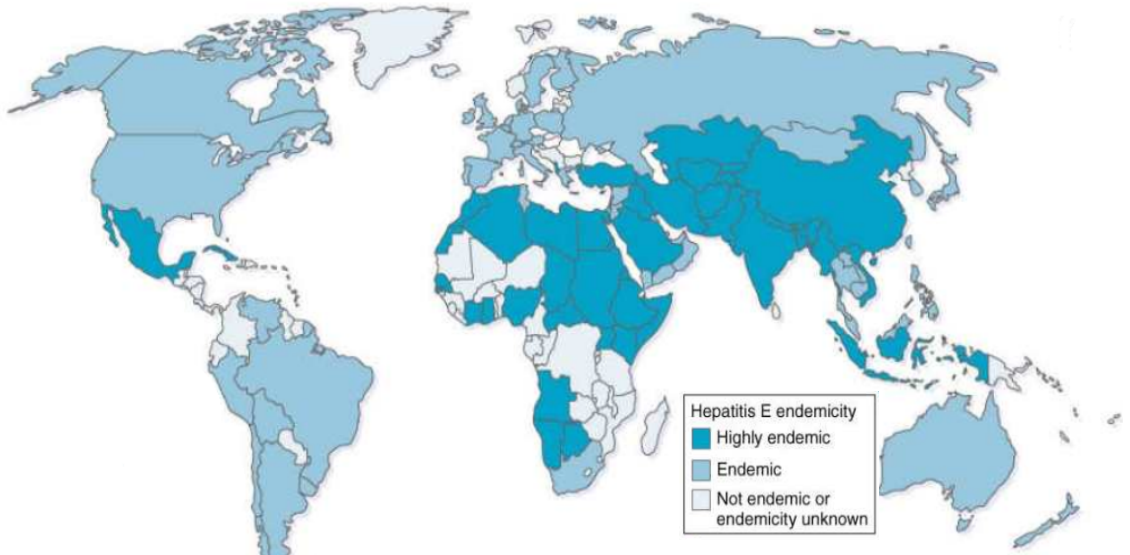
Epidemiology of HEV

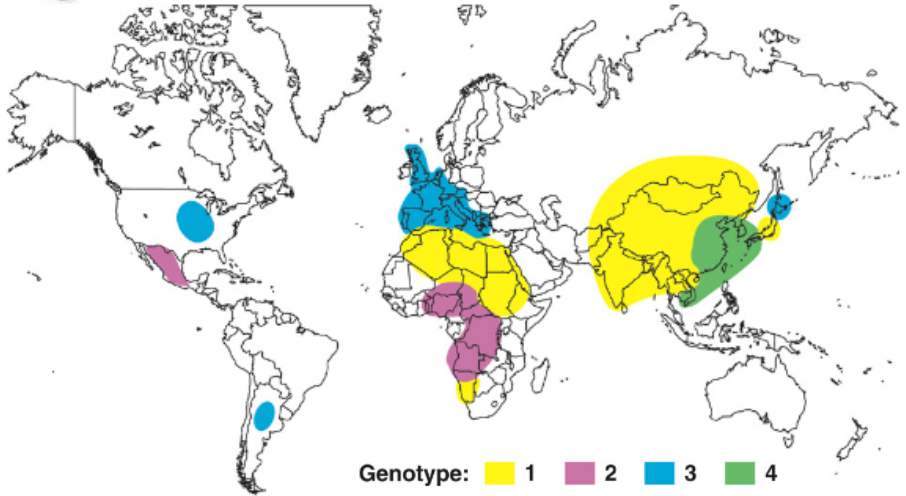
A worldwide diffusion

Global distribution

Two patterns:

- resource-poor area frequent water contamination
- areas with safe drinking water supplies





Aggarwal R. and Gandhi S., 2010, *Immunization, Vaccines and Biologicals,* WHO/IVB/10.14

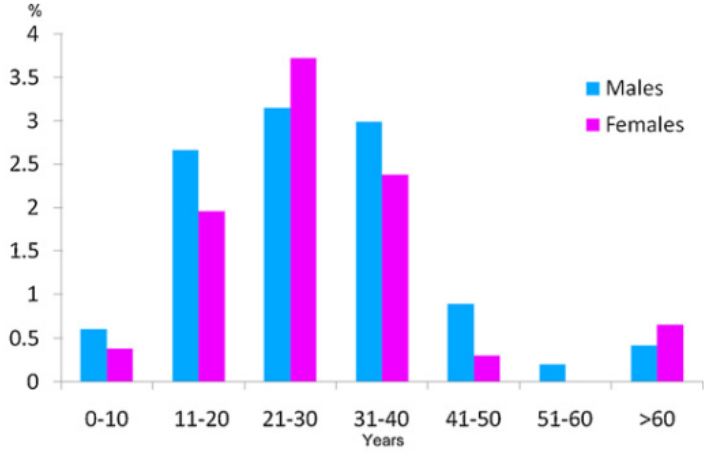
HEV in hyperendemic regions

- In hyperendemic regions, outbreaks occur frequently, usually separated by a few years
- Outbreaks are often large, and affect several hundred to several thousand persons
- Time-course varies from unimodal outbreaks, which last a few weeks, to prolonged, multipeaked epidemics which last for over a year
- Outbreaks frequently follow heavy rainfall and floods or happen in hot and dry summer months
- Few outbreaks related to food-borne transmission have been reported from hyperendemic areas

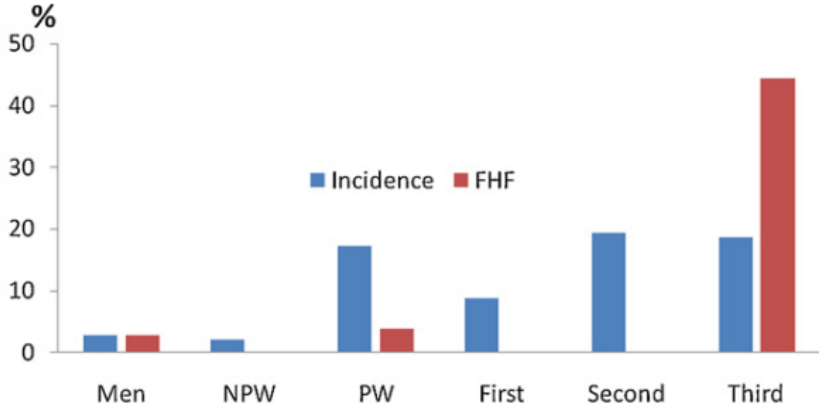
HEV in hyperendemic regions

- Overall attack rates during hepatitis E outbreaks have ranged from 1% to 15%

Kashmir epidemic-1978. Age and sex distribution in 275 cases of hepatitis E
recorded over six month period survey



Kashmir epidemic-1978. Incidence and fulminant rates of icteric hepatitis
E in pregnant women, nonpregnant women and men



M.S. Khuroo, Virus Research *161 (2011)* 3–14

HEV in low endemic regions

- Until a few years ago, most cases were found to be related to travel to disease-hyperendemic areas
- In recent years, solitary cases or small series of cases related to autochthonous transmission of hepatitis E have been recorded in USA, Europe and developed Asian–Pacific countries
- In these areas, zoonotic transmission appears to play a major role in transmission of hepatitis E virus

Surveillance

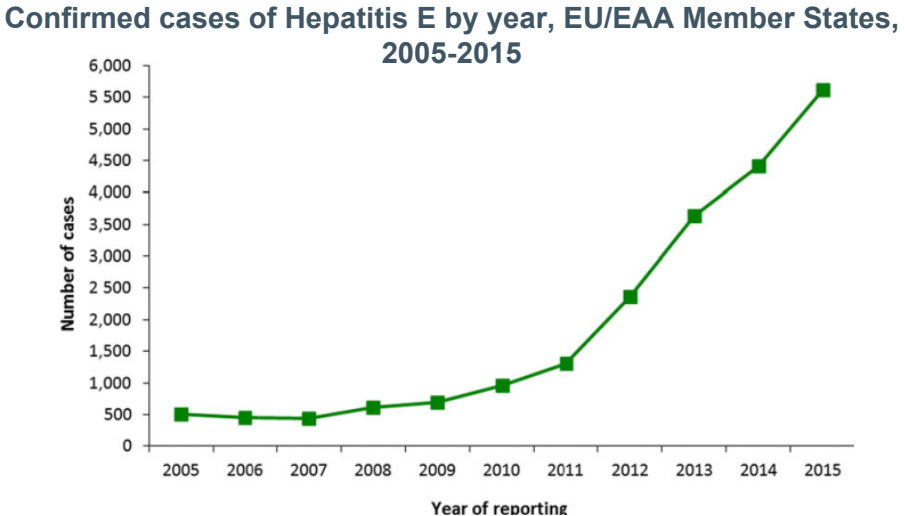
The EU/EEA situation

Surveillance systems

- HEV is not notifiable at the European level, but notification requirement may be present in individual MS
- 20 countries of EEA have a national HEV-specific surveillance established, 10 have a generic viral hepatitis syndromic surveillance in place
- Since 2007 Italy has a HEV specific surveillance program with a system of report to the National Health Service made by clinicians

Reported cases in EU/ EAA

- Overall more than 21.000 cases have been reported from 22 countries between 2005 and 2015
- The reported HE clinical cases increased from 514 in 2005 to 5.617 case in 2015

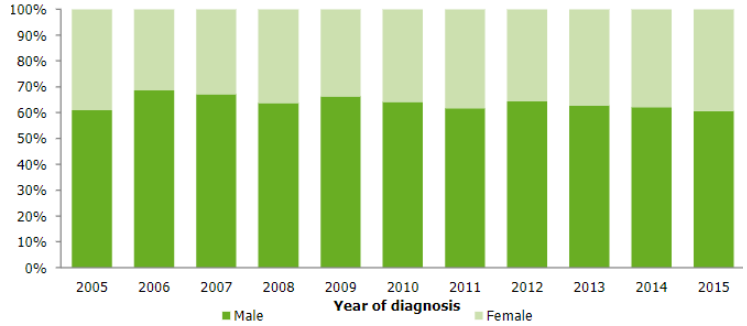


EFSA Journal 2017;15(7): 4886

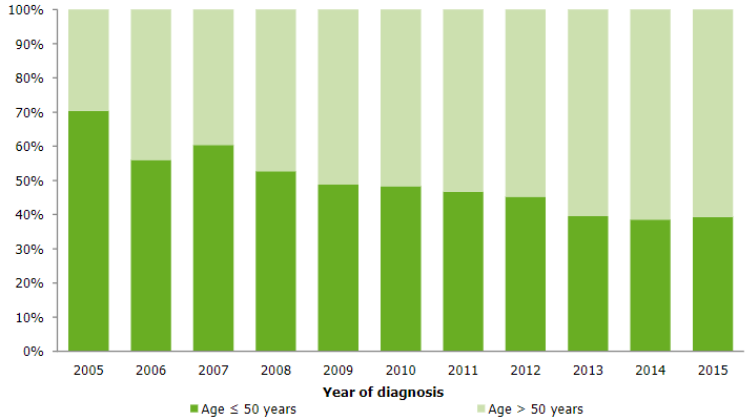
Reported cases in EU/ EAA

- Between 2005 and 2015 between 61% and 68% of all reported cases within EU/ EEA were reported to be male
- The proportion of cases being 50 years and older increased over the last 10 years from 30% to 61% in 2015

Proportion of confirmed cases by gender and year of diagnosis, EU/EEA Member States, 2005–2015



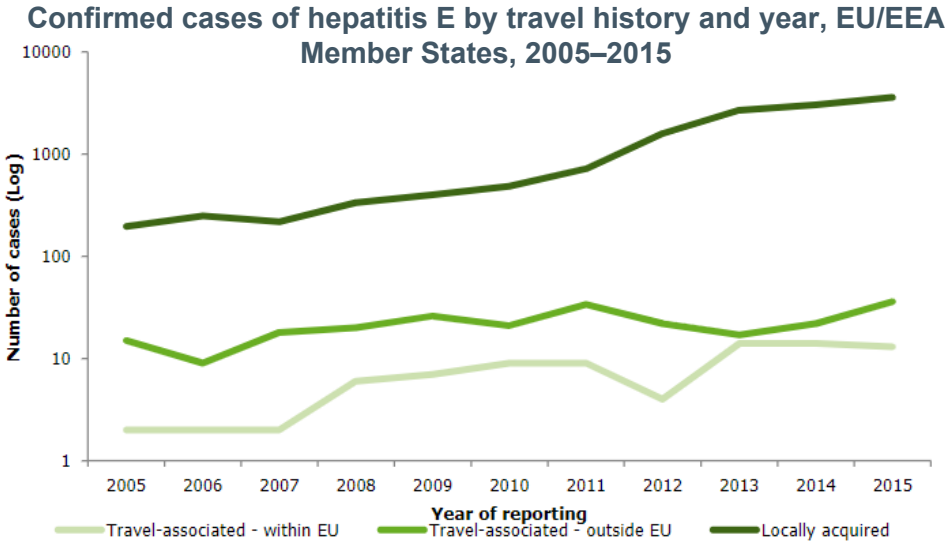
Proportion of reported cases aged ≤ 50 or > 50 years, by year of diagnosis, EU/EEA Member States, 2005–2015



ECDC Surveillance Report, Hepatitis E in the EU/EEA, 2005–2015

Reported cases in EU/ EAA

- More than 95% of the human infections were autochthonous, locally acquired (within the country of residence) due to infection with HEV-3 viruses
- Eating pork or wild boar products, raw pig liver sausages or other processed pork products were the main risk factors for HEV infection

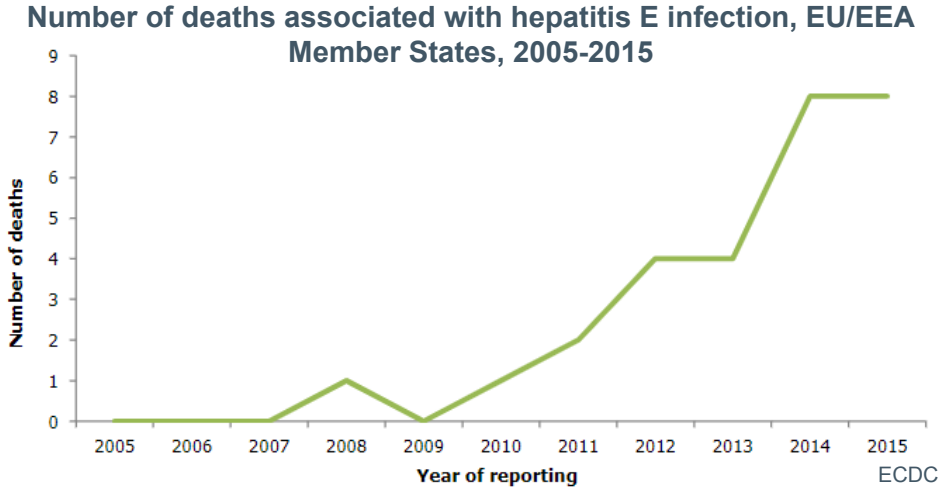


- Vegetarians generally have lower seroprevalence
- No direct human-to-human transmission has been observed in the EU, not even in the refugee camps where faecally contaminated drinking water was the source of infection

ECDC Surveillance Report, Hepatitis E in the EU/EEA,

Reported cases in EU/ EAA

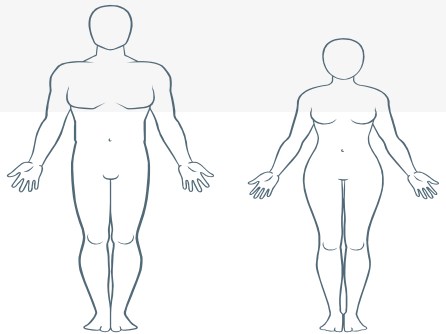
- Twelve countries, including Italy, reported a total of 28 deaths associated with HEV
- The number of deaths increased from 0–1 cases per year between 2005 and 2008 to 4–8 cases between 2012 and 2015



ECDC Surveillance Report, Hepatitis E in the EU/EEA,

HEV infection and disease in humans

In Europe, HEV is considered as an acute self-limiting hepatitis(70% infections are asymptomatic and people only seroconvert), involves mostly young adults aged 15 – 40 years.



HEV acute infection

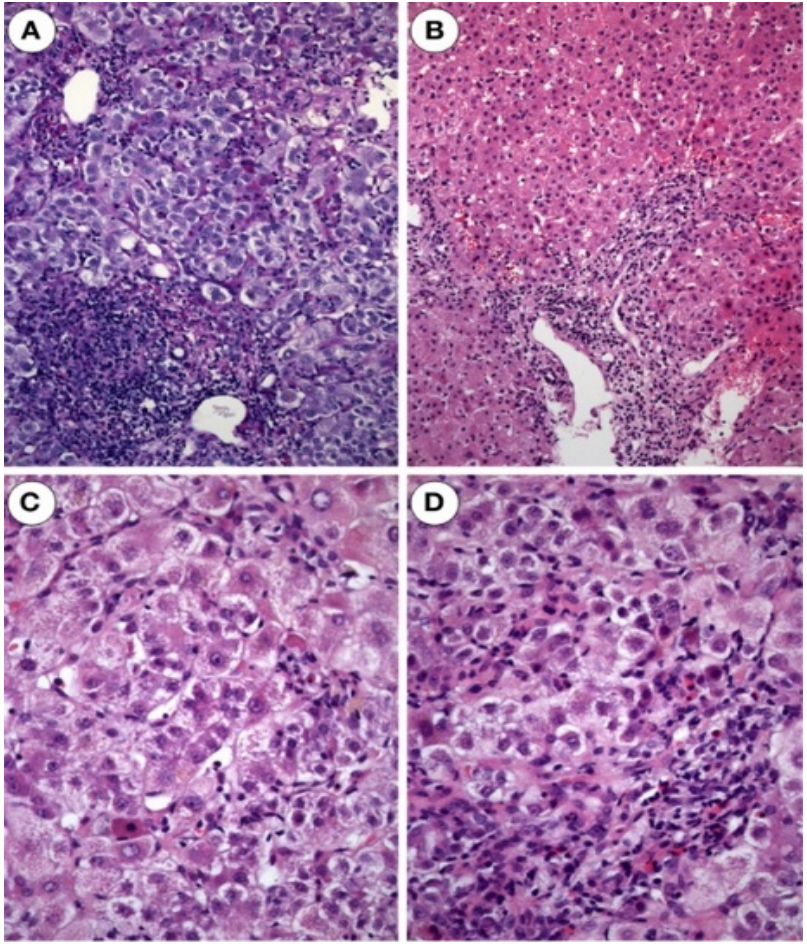
Symptoms are often indistinguishable from other liver illnesses .

- **The first incubation period** (asymptomatic): last about 2 - 6 weeks. Increase in serum transaminases already before symptoms.
- **The second period** ("pre-itteric"): non-specific symptoms, with flu-like symptoms (fatigue, mild fever, nausea, skin rashes or joint pain, followed by abdominal pain , vomiting, anorexia, malaise and hepatomegaly).
- **The third period** ("jaundice"): the complete illness phase, yellow discoloration of the skin and sclera of eyes , along with dark urine and pale stools. Patients have bilirubinuria, bilirubinemia, elevation of liver enzymes (AST, ALT). Prodromal symptoms tend to rapidly fade and eventually disappear. At the palpation of the abdomen hepatosplenomegaly and pain is appreciate.
- **The fourth period** ("convalescence"): general malaise and asthenia and abnormal liver functions may persist. With regression of the disease, the hematochemical (blood) values gradually return to normal. Clearance of infection is usually observed within 1–5 weeks

Histological aspects

Some aspects different from other forms of acute hepatitis.

- **Cholestatic hepatitis**: canalicular biliary stasis and "pseudo-glandular" alteration of parenchymal cells , with degenerative changes in hepatocytes
- **Balloniform cells** (degenerate hepatocytes) **and focal or confluent hepatocyte necrosis**. Focal lesions are similar to those of drug-associated hepatitis (accentuated cholestasis with bile accumulation)
- **Lobular inflammatory infiltration** (mainly macrophages and lymphocytes); the inflitrate is made of polymorphonuclear leukocytes.
- **Hypertrophy and hyperplasia of Kupffer cells and perisinusoidal cells**, while in portal spaces there is mixed inflammatory infiltrate.

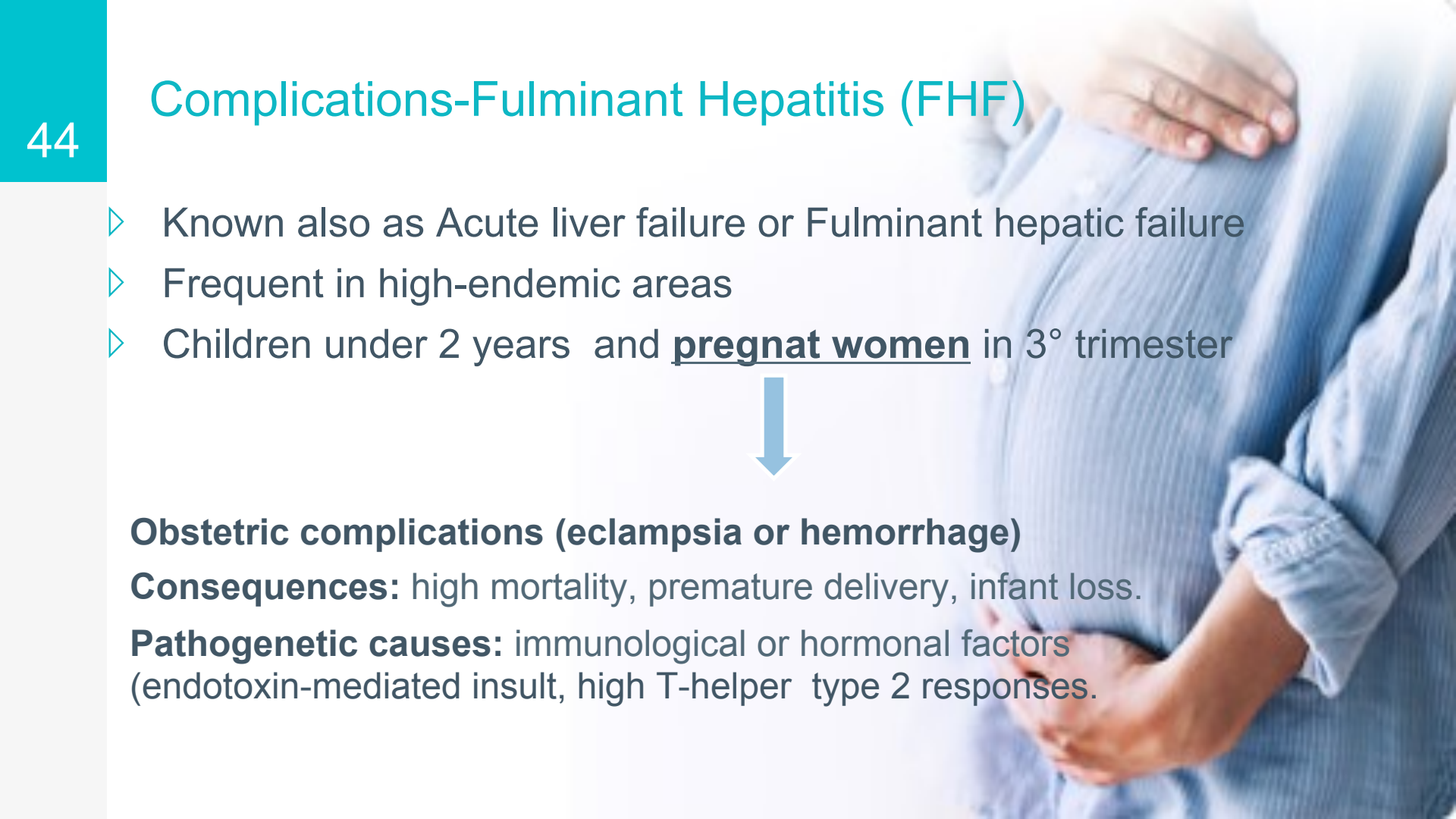


- **(A)** Expanded portal tract with dense inflammatory infiltrates mostly lymphocytes
- **(B)** Enlarged portal tract densely infiltrated by lymphocytes and some PMN leukocytes, some **spotty necrosis** in the lobule
- **(C)** Areas of spotty necrosis, aptotic bodies and **infiltrates of lymphocytes**, Kupffer cells
- **(D)** The lobule shows foci of spotty necrosis, **ballooning of hepatocytes** and infiltrates with lymphocytes and PMN

Drebber, Uta et al. “Hepatitis E in liver biopsies from patients with acute hepatitis of clinically unexplained origin” Frontiers in physiology vol. 4 351. 13 Dec. 2013

Complications-Fulminant Hepatitis (FHF)

- Known also as Acute liver failure or Fulminant hepatic failure
- Frequent in high-endemic areas
- Children under 2 years and **pregnat women** in 3° trimester



Obstetric complications (eclampsia or hemorrhage)

Consequences: high mortality, premature delivery, infant loss.

Pathogenetic causes: immunological or hormonal factors (endotoxin-mediated insult, high T-helper type 2 responses.

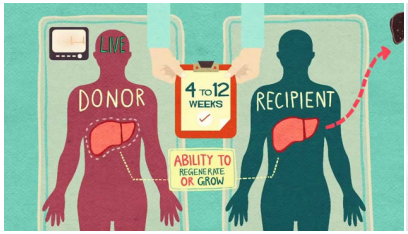
Chronic HE and HEV in immune-compromised patients

Persistent virus observed **for more than 6 months** (prolonged viremia)

Limited or non-specific clinical symptoms of hepatitis

Can develop rapid liver cirrhosis with a fatal outcome

Patients at risk:



- solid-organ **transplantation** / **trasfusions**
- **pre-existing liver disease**
- **haematological malignancy**
- **immunosuppressive conditions** (HIV patients have compromised immune system, and some but not all studies show higher HEV seroprevalence, particularly among HIV-infected patients with low CD4 cell counts or cirrhosis)

Extrahepatic manifestations of HEV in humans

Hepatitis E infections can have also extrahepatic manifestations in solid organ or bone marrow transplant recipient.

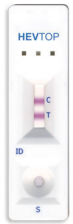
- **Neurological disorders**: such as Guillain-Barré, Turner syndromes, Neuralgic amyotrophy, Bilateral brachial neuritis, Peripheral neuropathy and Encephalitis
- **Organ injuries:** Renal lesions such as membranoproliferative glomerulonephritis, acute pancreatitis, autoimmune manifestations such as myocarditis, arthritis and thyroiditis
- **Haematological disorders:** Thrombocytopaenia

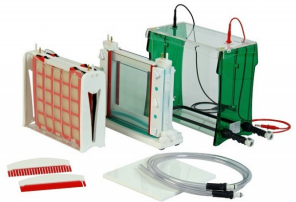
Viral-dependent complications: HEV-RNA detected in the LCR of patients with chronic infection.

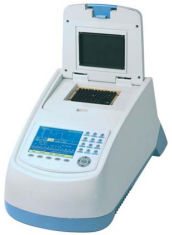
Viral clearance means complete resolution of neurological symptoms and significant improvement

Methods of detection of HEV in human samples

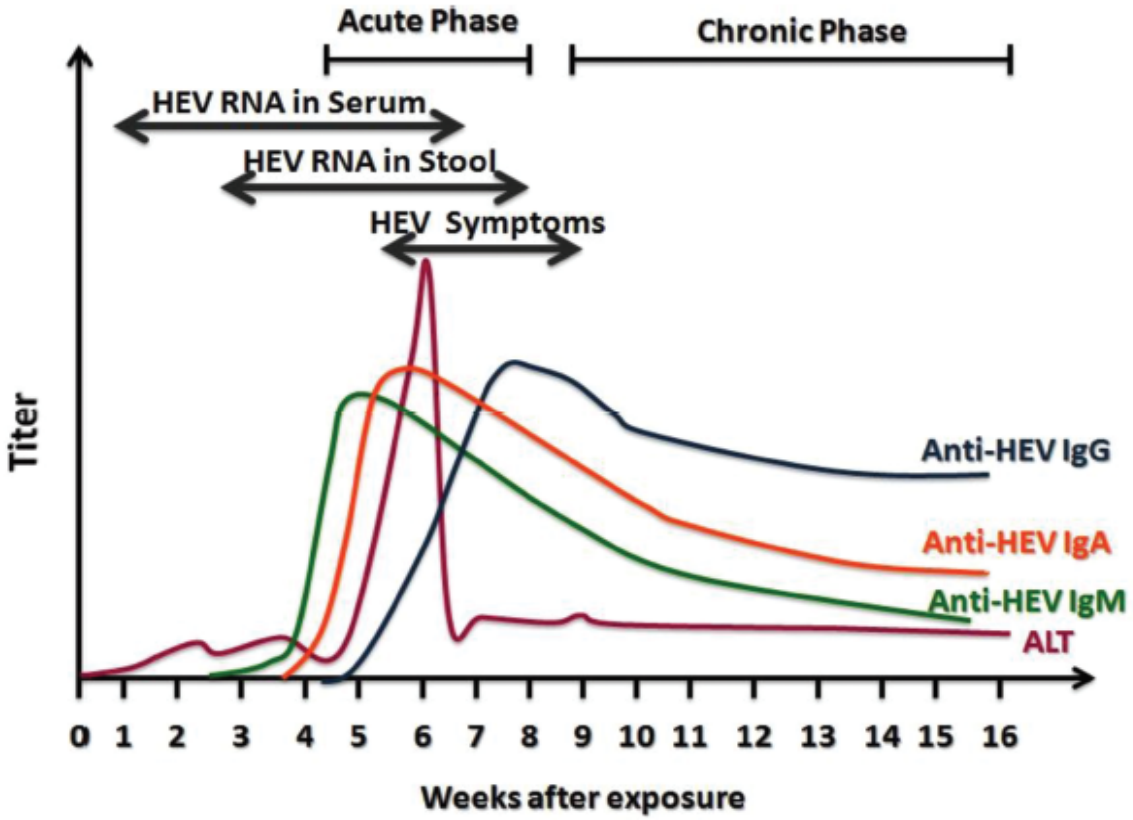
There are several techniques that can be used to detect HEV antibody, viral proteins and/or genome in patient samples.







Pathogenetic events troughout the course of HEV infection



- **RNA** can be detected very early in acute infection, decline in serum 5-6 weeks. Starts to appear in stool 2 weeks post infection (detectable only in acute phase).
- **IgM** are the first Ab produced and may persist until 6 month post infection.
- **IgA** are produced after and last about 1 month.
- **IgG** are produced after seroconversion and persist longer.
- **ALT** is a liver injury enzyme marker.

Diagnosis of HEV infection

Clinical
Patient examination

Laboratory
Serum / stool sample

Anamnesis

- **Travels** to endemic areas
- **Ingestion** of food at risk (fecal-oral route)
- **Close contact** with reservoir animals and same clinical signs among cohabitants.
- **Symptoms -** jaundice, hepatomegaly

Direct diagnosis

Virus / proteins

- **Detection of HEV** – immune electron microscopy, ISH,IHC
- **Detection of nucleic acid** – molecular method (RT-PCR, real time RT-PCR, LAMP, TMA, NGS)
- **Detection of viral protein/antigen** – enzyme immunoassay EIA

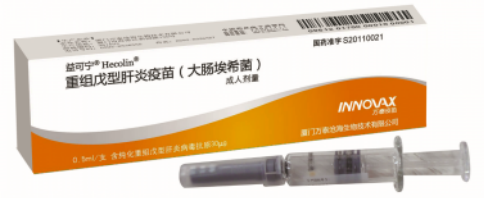
Indirect diagnosis

Host immune response

- **Detection of specific Ab isotype** – IgM (recent infection), IgG (past exposure), IgA (unclear).
  Sierological methods -Indirect EIA, ELISA, Immunochromatographic assay, Immunoblot assays

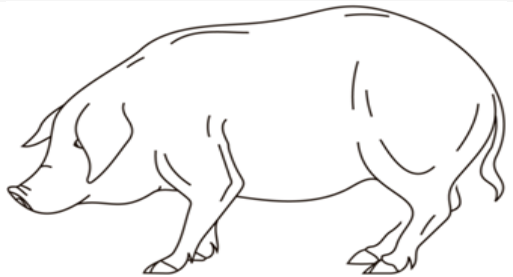
TREATMENT AND VACCINATION IN HUMANS

- No specific treatment, hospitalization not required (only in FHF patient and pregnant women)
- Antiviral therapy for risk groups (immunosuppressed, pre-existing liver disease) with reduction of immunosuppressive treatment + ribavirin or pegylated interferon-alpha
- HEV recombinant vaccine Hecolin (december 2011 only in China has been licensed)
- Based on ORF2 peptide
- High efficacy rate,
- Long term efficacy, duration and protection to be determined

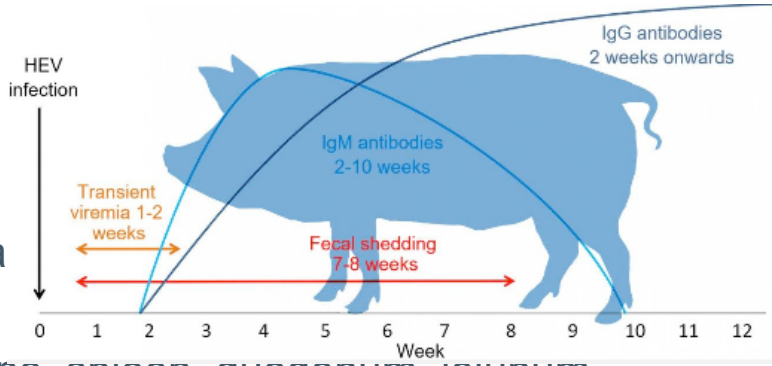


HEV infection in animals

Anti-HEV antibodies have also been detected in a large range of animal species, including pigs, wild boars, deer, moose, rats, dogs, cats, mongooses, cows, sheep, goats, avian species, rabbits, bats and horses.



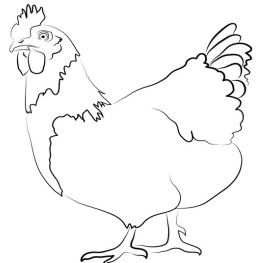
PIG S



- Strains related to human cases in industrialized a for which the source of infection was unknown.
- HEV has been found in liver but also in the intestine, spleen, duodenum, jejunum, colon, lung, gastro-hepatic lymph nodes and muscle
- Viremia last about 2 weeks, but is detected longer in stool samples (3/4 w.); seroconversion is evident 2 - 3 weeks p.i.
- **Subclinical infections** with only **histological signs** - periportal lymphoplasmocytic infiltrates, focal areas of vacuolization and hepatocellular necrosis.
- Presence of **anti-HEV Ab in most pigs above 3 - 4 months of age** (loss of maternal Ab); pigs below 2 months of age seronegative, and over 6 to 7 months renegativization but possible reinfection is not excluded (the virus in the pig may behave similar to that of hepatitis A in humans, which is pathogenic only for adults and not in young people).

POULTRY

- Discovered in 2001, a new HEV animal strain, causes poultry Hepatitis - **splenomegaly syndrome**
- Antigenically related to known human and pig strains and to an avian virus present in Australia - Australian chicken big liver and spleen disease virus (BLSV).
- Causes an **increase in mortality rates** (30 -72 weeks of age), and a decline in deposition at 20%, lesions such as **ovarian regression**, **hepatosplenomegaly**, **hepatic necrosis**, presence of **hemorrhagic fluid in the abdominal cavity**.
- Even the avian stock has the ability to "**jump**" the species barrier; has been proven that it can infect the turkey.



Methods of detection, quantification and typing of HEV in food and food-producing animals

The Subcommittee 'Microbiology' of the **International Organisation for Standardisation** (ISO/TC34/SC9) launched an enquiry in April 2015 for the development of a standardised ISO method for HEV detection in food samples in the future.

Molecular methods - RNA extraction from food and water

Essential to release the virus from samples before detection steps

Meat products (liver sausages):

- Homogenize sample with stomacher/ zyrconia beads, disruption in lysis buffer, centrifugation, polyethylene glycol (PEG) precipitation, chloroform-butanol treatment and magnetic immunobead-based RNA extraction

Soft fruit or leafy green vegetables:

- Pectinase treatment, ultrafiltration, PEG precipitation, silica-based columns RNA extraction

Shellfish (digestive glands):

- PEG precipitation, ultracentrifugation, ultrafiltration or ethanol precipitation

Environmental waters (Irrigation, drinking water, start Vol 50 mL):

- Filtration methods – use of +/- charged filter membranes, glass wool ultrafiltration, flocculation at low pH

Standardisation, systematic method comparison, interlaboratory validation have not been performed for most virus extraction methods.

Detection and quantification of the HEV genome - nucleic acid amplification techniques (NAT)

1.End point RT-PCR

-target several regions of HEV genome (primers - terminal part of ORF2)

-electrophoresis gel detection

-susceptibility to contamination

-low sensitivity

2.Real time RT-PCR (multiplex, digital, nested)

-higher sensitivity and specificity

-faster performance

-fluorescent detection

-quantification can be done if standards (ampl. Curves) are used on specific software

3.Loop mediated isothermal amplification (LAMP)

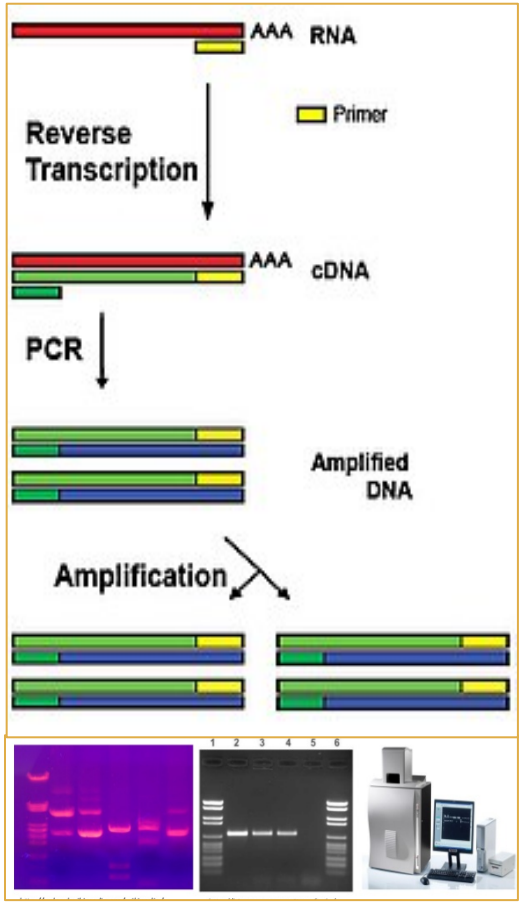
-high sensitivity and efficacy, use a set of 6 primers

-no need of thermocycler,

only one-step of amplification at isothermal conditions (63°C for 60’)

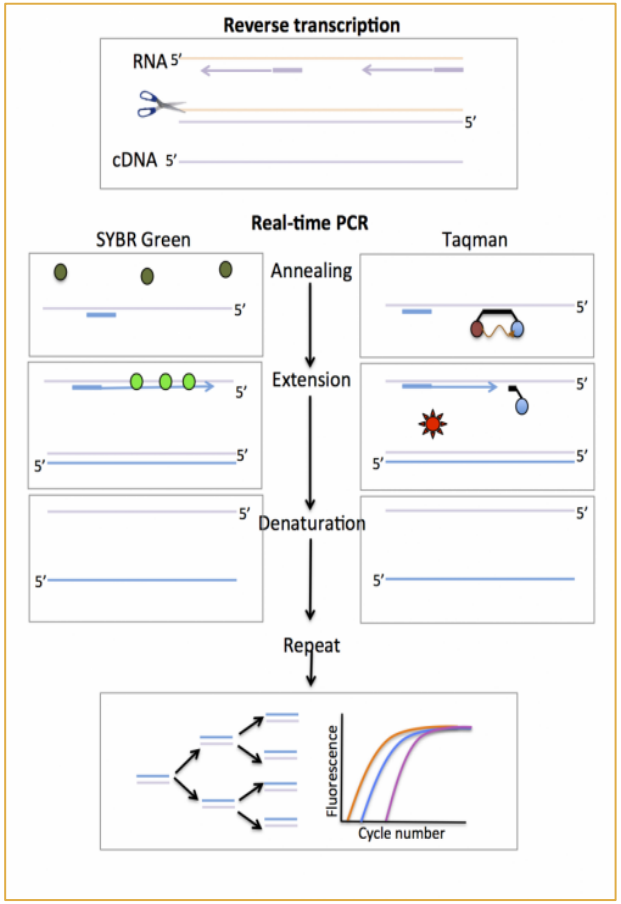
-reaction interpretation with turbidity

1.



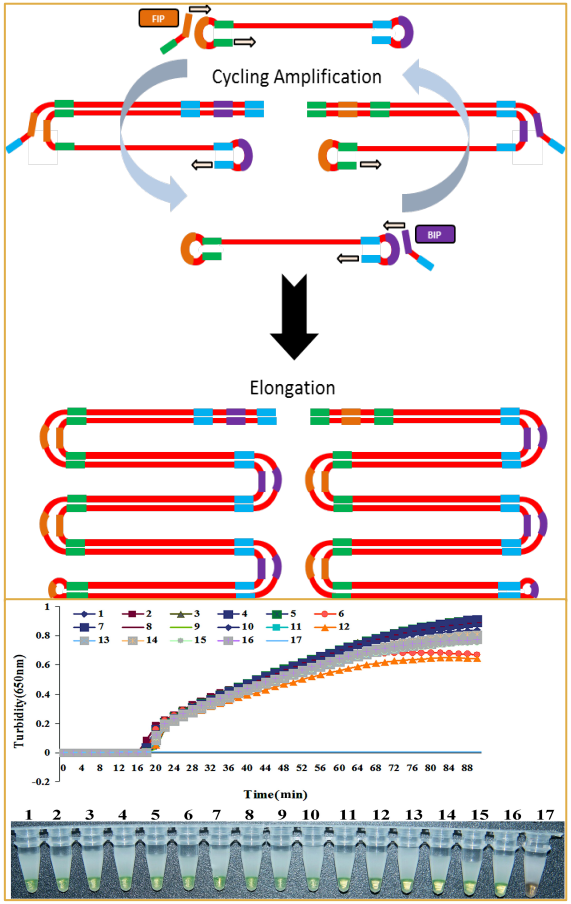
Result detection - Electrophoresis

2.



Fluorescent probes – esponential phase detection

3.



Precipitation, Result detection- turbidity

Typing methods of HEV strains, comparison to reference strains

Due to various factors such as the high **diversity** and the **plasticity of the RNA genomes**, accurate **typing** of HEVs remains a **challenging task.**

Sensitive and rapid alternative for the classification of HEVs.

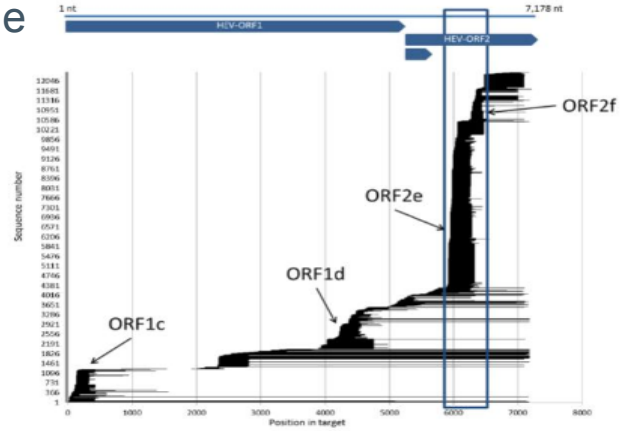


Figure 2: Distribution of sequences available in the GenBank (April 2016) database along the HEV genome (Courtesy of H. Vennema, RIVM)

- Molecular techniques such as **RT-PCR** in conjunction with **sequence alignment** reconstruction **algorithms**, nucleotide sequence **distance calculation, phylogenetic tree construction**, **comparison** with previously typed strains.
- **ORF1c**, **ORF1d**, **ORF2e** and **ORF2f** regions (specific gene shared by HEV genomes)
- European Centre for Disease Prevention and control (ECDC) has established an **expert group of national public health epidemiologists** and **virologists** to review the epidemiological situation of HEV in the European Union (EU)/EEA Member States (MS) and to **develop a web-based sequence** store that can be a **genotyping and subtyping tool** for HEV strains (‘**HEVnet’**), by collecting and

Hepatitis E Virus Genotyping Tool Version 0.1

This tool is designed to use phylogenetic methods in order to identify the Hepatitis E virus genotype of a nucleotide sequence.
Note for batch analysis: The genotypetool accepts up to 20000 sequences at a time.

You may either:

A. Paste one or more sequences in FASTA format in the input field.
B. Upload a FASTA file.
C. Upload FASTQ files.
D. Revisit results of a previous run

A) Paste nucleotide sequence(s) in FASTA format:

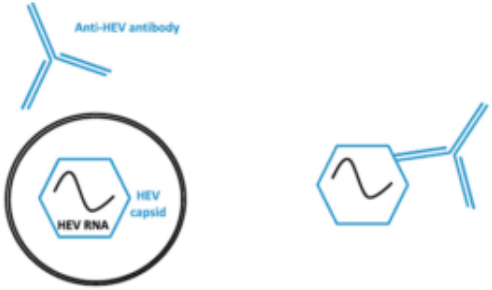
```
>AB222087|HepE3
AGGTATGGCCGTAGAACGAAATTATATGAGGCGGCACATTCAGATGTTCGTGAGTCCCTAGCTAGGTTCATCCCCACTATCGGGC
CTGTTCAGGCCACCACATGTGAGTTGTATGAGTTGGTTGAGGCCATGGTGGAGAAGGGTCAGGACGGCTCTGCCGTCCTAGAGCT
TGACCTTTGCAATCGTGACGTCTCGCGTATCACATTTTTCCAAAAGGATTGCAATAAATTCACAACTGGTGAGACTATTGCCCAT
GGCAAGGTTGGCCAAGGTATATCGACCTGGAGTAAGACCTTCTGTGCCCTGTTTGGCCCGTGGTTCCGCGCCATAGAAAAAGAGA
TATTGGCCCTGCTCCCGCCTAATATCTTTTATGGCGACGCTTATGAAGAGTCAGTGTTTGCTGCCGCTGTGTCTGGGGCGGGGTC
ATGTATGGTATTTGAAAATGATTTTTCGGAATTTGACAGTACTCAGAATAACTTCTCTCTCGGCCTTGAGTGTGTGGTCATGGAG
GAGTGCGGCATGCCCCAGTGGTTGATTAGGTTGTACCATCTGGTTCGGTCAGCCTGGATTTTGCAGGCGCCGAAGGAGTCTCTTA
AGGGTTTTTGGAAGAAGCACTCTGGTGAGCCTGGTACCCTTCTCTGGAACACCGTCTGGAACATGGCGATTATAGCGCACTGTTA
CGAGTTCCGTGACTTTCGCGTTGCCGCCTTCAAGGGTGACGATTCGGTGGTCCTTTGCAGCGACTATCGGCAGAGCCGCAATGCG
GCTGCCTTAATTGCAGGCTGTGGGCTCAAATTGAAGGTCGATTATCGCCCTATTGG
```

Start! Clear

Metagenomic identification of HEV, next-generation sequencing (NGS)

- **High-throughput sequencing methodology** that generates millions of sequences simultaneously from one sample. New perspectives for virus research and diagnostics.
- Most used platforms; **Roche 454 pyrosequencing and Illumina**
- The latest NGS systems (singlemolecule real-time technology (SMRT)) are still in the evaluation stage - soon commercially available.
- The use of NGS techniques such as metagenomics should be considered for the **characterisation of a community of contaminant viruses** or **identification of new viral strains** (novel virus discovery) in different hosts, food or environement, but further development is needed to improve the sensitivity
- The applications of NGS allow **reconstruction of full-length viral genomes** (whole genome sequencing).

Antibody-based assays



- **Recognition of the specific immune response of the host organism against the causative agent**. In food-producing animals , the methods depend on detection of anti-HEV Ab against HEV-3, HEV-4 but the availability of commercial assay is limited.
- **IgM** anti-HEV - marker for recent exposure. **IgG** anti-HEV - sign of prior HEV infection (estimate the exposure to HEV in a population over a period of time). **IgA** role in immune function of mucosas membrane.
- In pigs and other animal species detection can be performed by three methodologies:

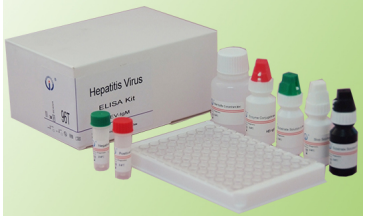
(1) **commercially available assays for Anti-HEV Abs** (enzymelinked immunosorbent assay ELISA, westernt blot WB, enzyme immunoassay EIA) for Abs detection in porcine serum;

(2) **commercial kits** optimised for detection of human anti-HEV Abs that have been **adapted** to use for **swine** or other animal species;

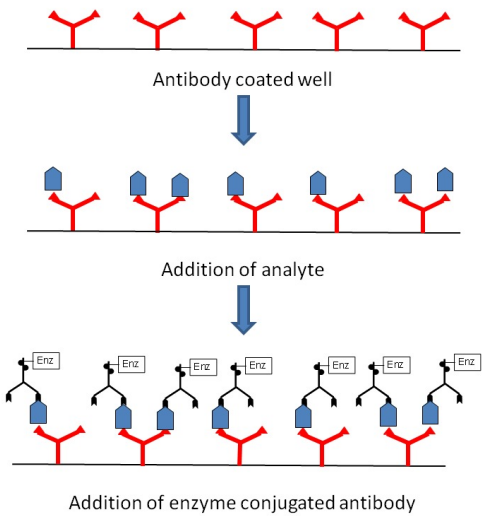
ELISA

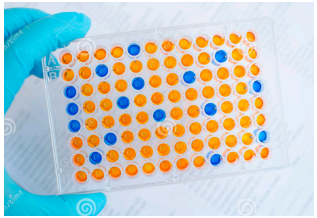
Test usually applied as a screening test in veterinary medicine

- Detection of anti-HEV Ab in porcine sera
- HEV-3- or HEV-4-related **ORF2** and **ORF3** (were expressed in Escherichia coli, yeast, baculovirus-infected insect cells or mammalian cells) **proteins** have been used as **coating antigens** or synthetic peptides or chimeric constructs harbouring multiple epitopes
- correlates well with quantitative detection of HEV RNA in matrices tested
- shows **limited sensitivity**



Sandwich ELISA





Visual changes indicates the presence of desired protein

Infectivity assays - Inoculation of animals

Use in vivo models: determination of the host range of HEV, clinical investigations, inactivation studies - rarely used

- Several **animal** species have been **tested for susceptibility to infection**: monkeys, pigs (only be susceptible to HEV genotypes 3 and 4, with no signs of disease), rabbits, gerbils, rats, mice and tree shrews.
- **Recently,** immunosuppressed mice repopulated with human liver cells have been developed as **chimeric model for infection studies with HEV-1 and HEV-3.**
- In these animal species, different **routes of infection** have also been tested, and these indicate that **intravenous infection is most efficient,** whereas oral infection usually failed.
- Limitations - **amount of virus** needed for infection of animals unknown, **ethical considerations**, restriction of **sample numbers** (experiments with large animals are laborious, time consuming and expensive)

HEV isolation - Cell culture

- **HEV is a difficult virus to be grown in vitro** but different studies describe isolation and propagation of HEV-1, HEV-2, HEV- 3 and HEV-4 in cell culture (first in 1996).
- HEV obtained from **clinical specimens such as serum, faeces** or **liver Homogenates** (low HEV concentration clinical samples = unsuccessful cell culture isolation)
- Human liver carcinoma cell lines **PLC/PRF/5 and HepG2/C3A** or the human lung carcinoma cell line **A549** most used. Slow replication and moderate titres in the culture supernatant, for this reason no cytopathic effect was evident. **RT-qPCR or immunofluorescence are necessary to detection virus replication.**
- HEV-3 strains **Kernow-C1 and 47832c,** isolated from **immunosuppressed transplant patients**, replicate more efficiently compared with other field strains (higher sensitivity of the 3D grown cells for HEV was shown)
- The development of efficient cell culture methods for HEV should be encouraged to facilitate acquisition of quantitative data on infectivity, inactivation and survival of HEV in food and in the environment.

Control strategies

Possible control measures along the food chain and evaluation of decontamination treatments

Control of HEV in the pork food chain

- Control methods for HEV in food will differ between commodities depending on the risk of contamination of the specific products
- The current control measures for food of animal origin rely on EU legislation but, to date, no specific legislation or specific measures for HEV exist
- There are currently no official control policies regarding HEV in animals

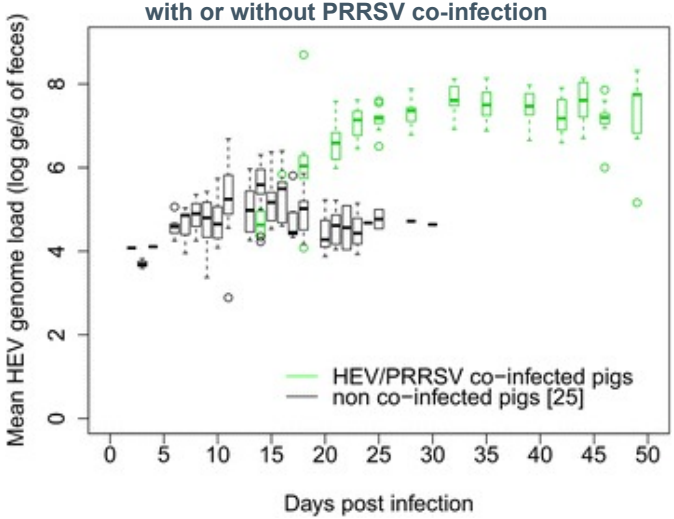
Control of HEV at farm level

- Aimed to prevent HEV introduction into pig breeding pyramids and production herds and thus reduce number of HEV-infected pigs at the time of slaughter
- Reducing the prevalence of infected pigs by appropriate farm management, hygiene (including effective disinfection of pig housing and equipment between batches) and biosecurity measures
- Use of vaccination could be a valuable control measure. Vaccine is licensed in China to be used in humans but so far there are non studies of its use in pigs

Control of PRRSV

- In an experimental HEV/PRRSV co-infection study in specific-pathogen-free pigs, follow-up of co-infected animals showed that HEV shedding was delayed by a factor of 1.9 compared with HEV-only infected pigs

Distribution of the number of HEV genome equivalent (log ge/g feces) shed by individual pigs with time in inoculated animals with or without PRRSV co-infection



Summary of the infectious dynamics parameters and comparison with data from the HEV-only infection experiment

	HEV + PRRSV	HEV alone [25]
Latent period (days)	13.4	7.1
	(8.6; 17.1)	(3.2; 12.3)
Infectious period (days)	48.6	9.7
	(27.9; 84.6)	(8.2; 11.2)
Seroconversion period (days)	43.1	26.3
	(35.7; 52.2)	(23.5; 29.5)
Direct transmission ($days^{-1}$) β_w	0.70	0.15
	$(1.2{\cdot}10^{-3}; 3.67)$	(0.03; 0.31)
Indirect transmission (g/ge/d) β_E^w	$6.6{\cdot}10^{-6}$	$2.0{\cdot}10^{-6}$
	$(1.4{\cdot}10^{-10}; 1.3{\cdot}10^{-4})$	$(1.1{\cdot}10^{-7}; 7.0{\cdot}10^{-6})$

- Identification of the specific immune response was delayed by a factor of 1.6
- Infectious period prolonged
- Transmission rate was estimated to be 4.7 times higher

Salines et al. Veterinary Research (2015)

Control at slaughterhouses, meat processing plants and retail

- Good practice during transport, lairage and at the slaughterhouse as well as during processing and storage should reduce the risk of HEV cross-contamination
- Apply skinning with hot steaming or burning
- Bunging and hot water washes may also be applied to minimise faecal contamination
- Blast freezing is increasingly common and may have some antimicrobial effect, but possibly less so against viruses than bacteria
- Dedicated equipment and utensils, particularly knives, should be used only for their specific operations. This rule is imperative when removing internal organs
- Knifes and cutting tools in robots should be treated with hot water (85°C for 15 s) after each operation

Control of hunted game

Hunters may have direct contact with blood or other body fluids if they do not wear any barrier protection, such as protective gloves. No specific microbiological criteria for game meat exist as yet in the EU legislation. General recommendations are:

- Wearing protective gloves during disemboweling and paying attention to abrasions on the skin
- Knives and other utensils for raw venison and offal should be used only for these purposes
- Hands should be properly washed after disembowelling and handling of wild boar, venison or their offal
- Hunters and others handling carcasses as well as the general public should be educated about HEV transmission associated with game mammals
- Sufficient heat-treatment of wild boar meat, venison and offal prior to consumption should be performed

Control of bivalve molluscs food chain

- Specific requirements for the hygiene of live molluscs are covered by specific regulations (ECs 852/2004, 853/2004 854/2004, 2073/2005)
- Legislative standards are based on indicator bacteria, E. coli or faecal coliforms which determines the classification of production areas: A (cleanest), B or C (most contaminated)
- Epidemiological and laboratory studies show that depuration times and conditions currently used are inadequate for HEV
- The main measures should be to prevent viral contamination of the molluscs by improving environmental conditions (particularly water quality) in production and harvesting areas

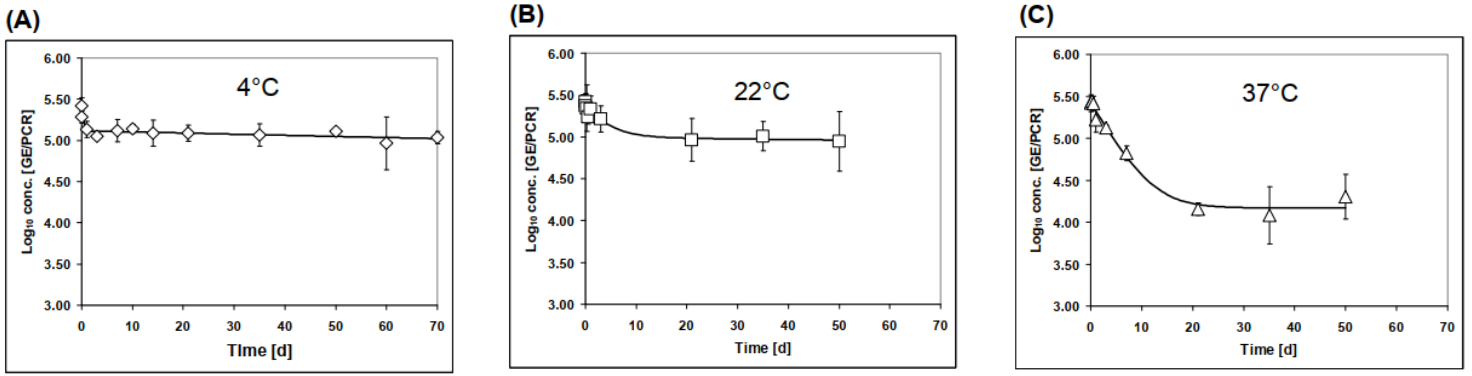
Control of fruit and vegetable food chains

- Limited information is available about the stability of HEV in the environment outside the host organism as well as its resistance to decontamination procedures
- General recommendations regarding the other food-borne viruses should be followed
- Selection of the water sources for irrigation and agricultural chemicals
- Disinfection of fruits and vegetables may only have limited effect on HEV

Effect of time–temperature combinations on HEV

- Data obtained by treatments of HEV at various combinations of temperature and time show a rapid initial decline of genome equivalent (GE or RGE) or focus forming unit (FFU) followed by a slower inactivation/reduction step
- HEV is stable under long-term storage conditions and remains infectious for several weeks at room temperature

Effect of long-term storage at different temperatures on HEV stability. An HEV-containing liver suspension was incubated at 4°C (A), 22°C (B) or 37°C (C) for up to 70 days and protected RNA was quantified using real-time RT-PCR.

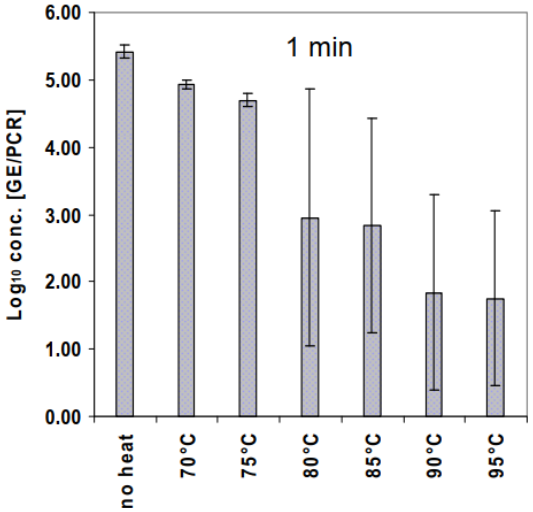


Schielke et al.,

Effect of time–temperature combinations on HEV

- Total inactivation was observed at higher temperatures, for example, higher than > 56°C for 1 h or a shorter time (1–2 min) but at higher temperature
- HEV resistance to heat treatments can also vary depending on the matrix (e.g. faeces, liver, percentage of fat)
- Virus embedded in a slab of meat is expected to require a higher temperature to be inactivated

Effect of short-term heating on HEV stability. The HEV-containing liver suspension was heated at different temperatures for one minute, and protected RNA was quantified using real-time RT-PCR. The columns represent mean values from three independent tests



Schielke et al., 2011; Rogee et al.,

UV

- Ultraviolet light can inactivate food-borne microorganisms without substantially heating the food, producing safer food and preserving high sensory and nutritional values
- UV radiation is, however, only suitable for decontamination of surfaces or thin layers of clear liquids
- Ultraviolet treatments of human HEV-3 viral suspension using low UV fluence result in a 99.99% reduction of infectivity
- A residual infectivity is always observed because virus can be embedded in cells resulting in a less exposure to the UV irradiation

Chlorine and other disinfectants

- The reduction of the infectious units of HEV depends on the dose used
- The efficacy of chlorine disinfection is influenced by several parameters, such as surface morphology, temperature and pH
- Alternatives to NaOCl are ClO_2, H_2O_2, peracetic acid (PAA), NH3, O_3
- Data for HEV are lacking, but results obtained for other enteric viruses may demonstrate potential virucidal activity against HEV

Hydrostatic pressure processing

- HPP shows great potential in the food industry for inactivating microorganisms while maintaining the quality of fresh foods
- Pressures between 400 and 600 MPa are applied, with time ranging from 3 to 30 min
- No information is available on reductions of HEV by HPP. However, several other enteric pathogenic viruses have been studied
- The efficacy of virus inactivation is not only dependent on the pressure, but also dependent on the pH and salt content of the matrix, the temperature and the strain of the virus

Irradiation

- Irradiation may cause a change of texture or colour in fruit and vegetable tissues
- Three different types of ionising radiations: gamma rays (c-rays), X-rays and electrons (e-beams)
- No specific data are available on inactivation of HEV
- In the EU, 12 MS have a total of 23 irradiation facilities approved for irradiation of food
- The foodstuffs included in this list that may play a role in transmission of HEV to humans include dried vegetables and pulses, soft fruit, chicken and poultry, shellfish
- Pig meat and other pig products are not listed in this EU regulation

THANKS

FOR YOUR ATTENTION

By:
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Giuseppe Zarola
Cristina Monica Cristinariu