



## Corso Precongressuale C

Epatiti virali stato dell'arte:  
screening, diagnosi, novità  
terapeutiche, farmacoresistenza  
e patogenesi

HEV: un virus da  
non sottovalutare

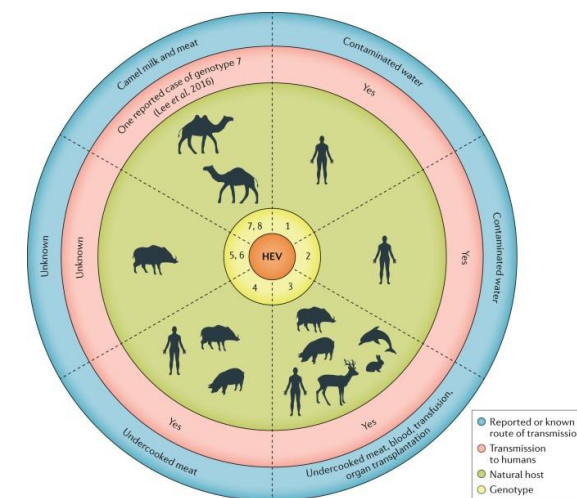
Maria R. Capobianchi



Istituto Don Calabria  
IRCCS Ospedale  
Sacro Cuore Don Calabria  
Presidio Ospedaliero Accreditato - Regione Veneto

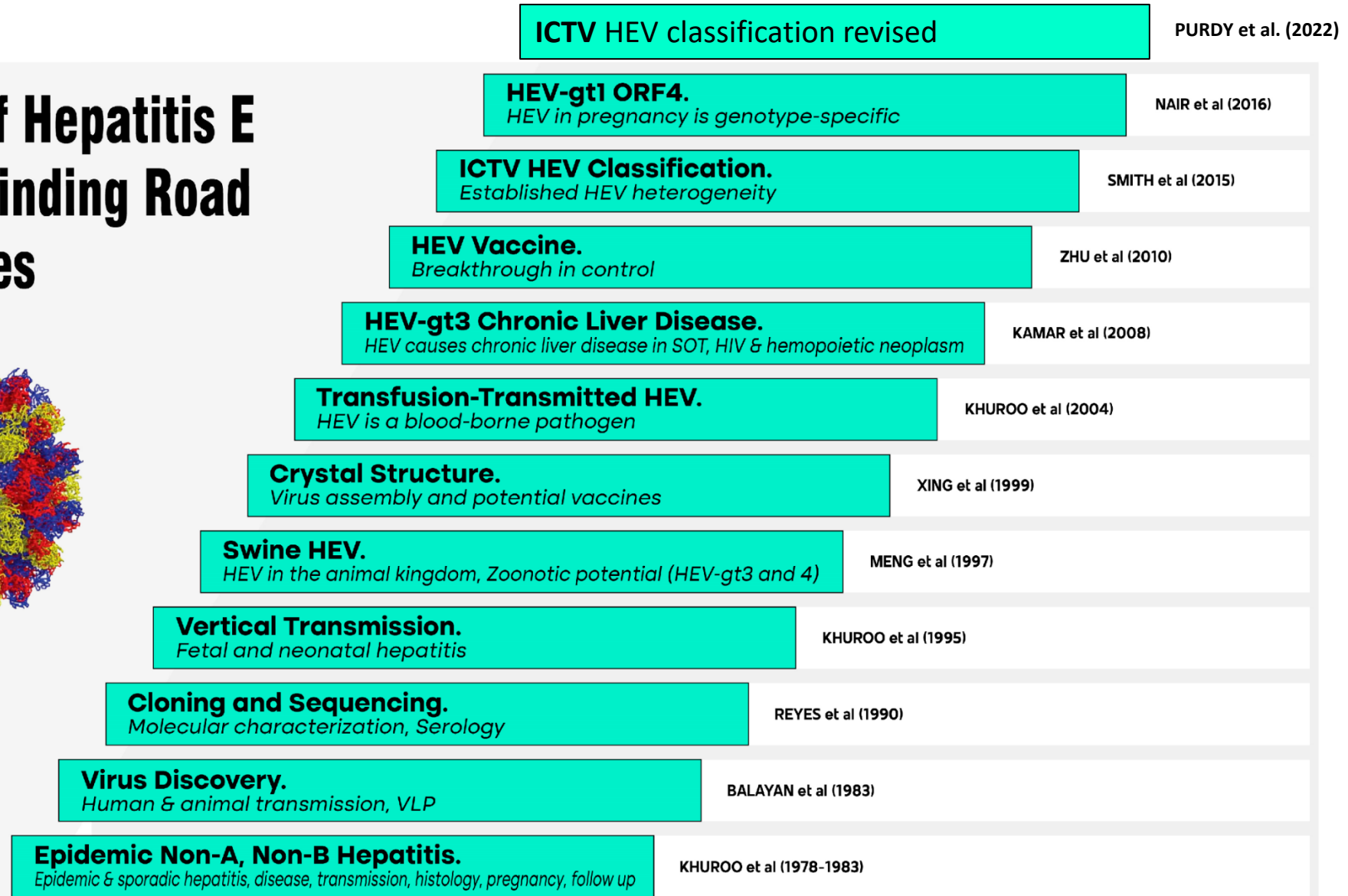
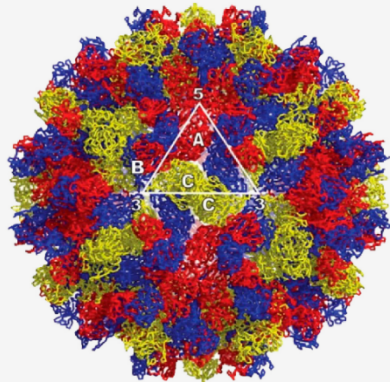


UNICAMILLUS  
International Medical University in Rome



## A Journey of 40 Years about an Incredible Story

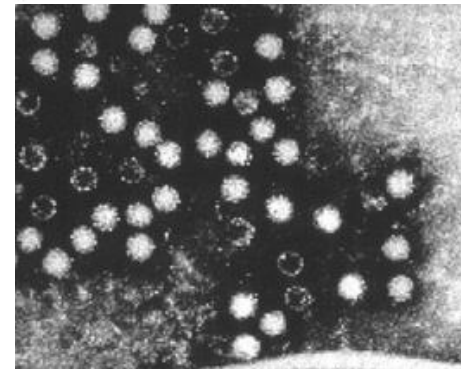
# Discovery of Hepatitis E Long and Winding Road of Milestones



## 1983: Mikhail Balayan and the Bizarre Discovery of Hepatitis E Virus

Dr. Mikhail Balayan

- In **1983** M. Balayan, from the Institute of Poliomyelitis and Viral Encephalitides, Moscow, USSR, was investigating an outbreak of non-A, non-B hepatitis in among Soviet soldiers in a military camp in Afghanistan.
- He wanted to bring patient samples back to Moscow, but he had no means for refrigerating the samples, and no permission from his supervisors to return with the samples.
- So, he solved the dilemma by a rather extreme experiment: he drank a pooled filtrate of patient stools (**mixed with kefir**).
- After returning to Moscow, on the 36th day after ingestion of the inoculum, he came down seriously ill with hepatitis.
- He began to collect his own stool samples, in which he detected, by electron microscopy, 32 nm virus particles, causing hepatitis when inoculated into monkeys. Balayan's virus looked like HAV in electron micrographs.
- He could show that it was not hepatitis A virus, since he already had antibodies against HAV.



# Outline

- HEV epidemiology: HEV is an emerging pathogen whose burden is largely underestimated due to underdiagnosis
- The biology of the virus (i.e. its quasi-enveloped nature) has been only recently partly elucidated
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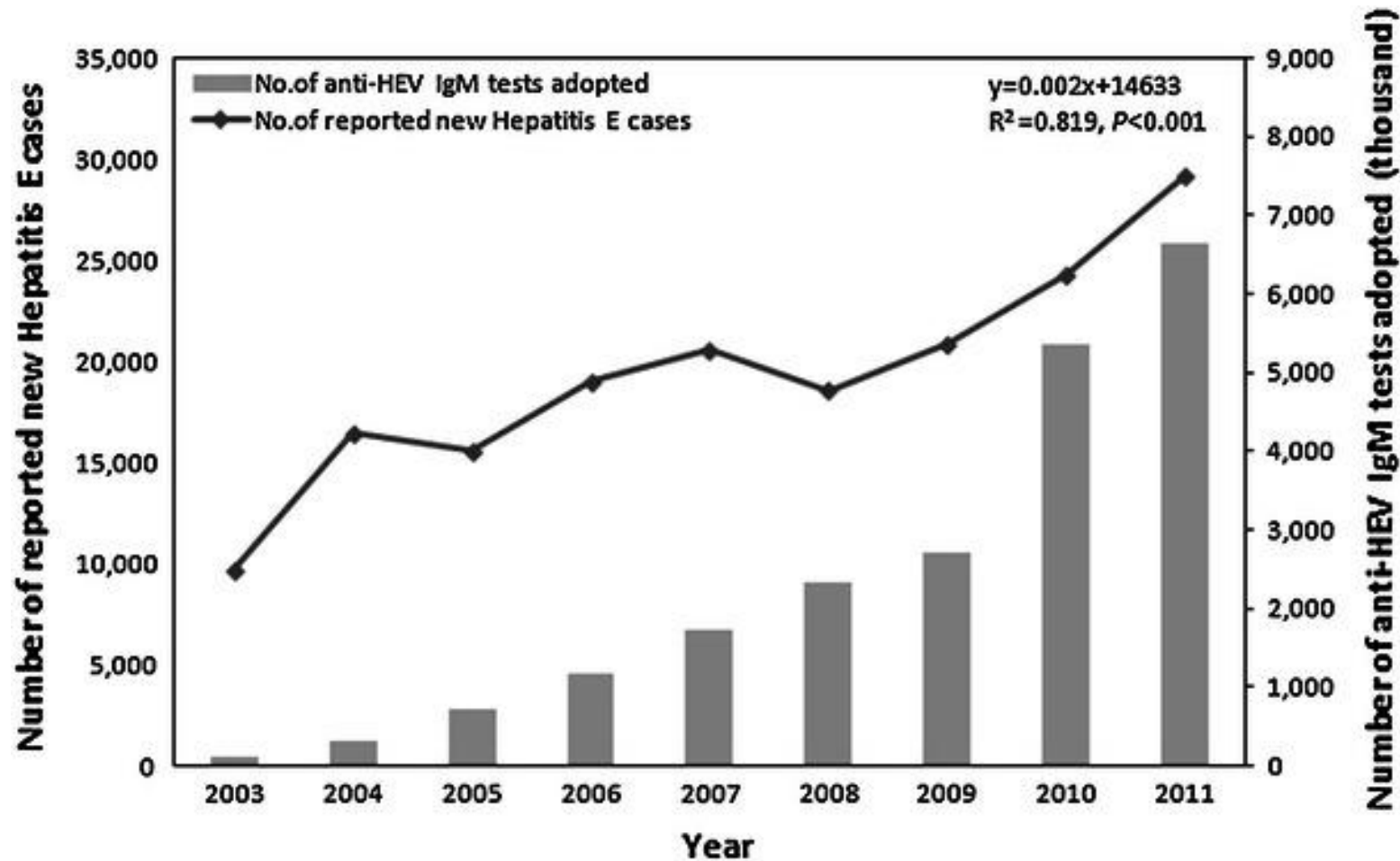


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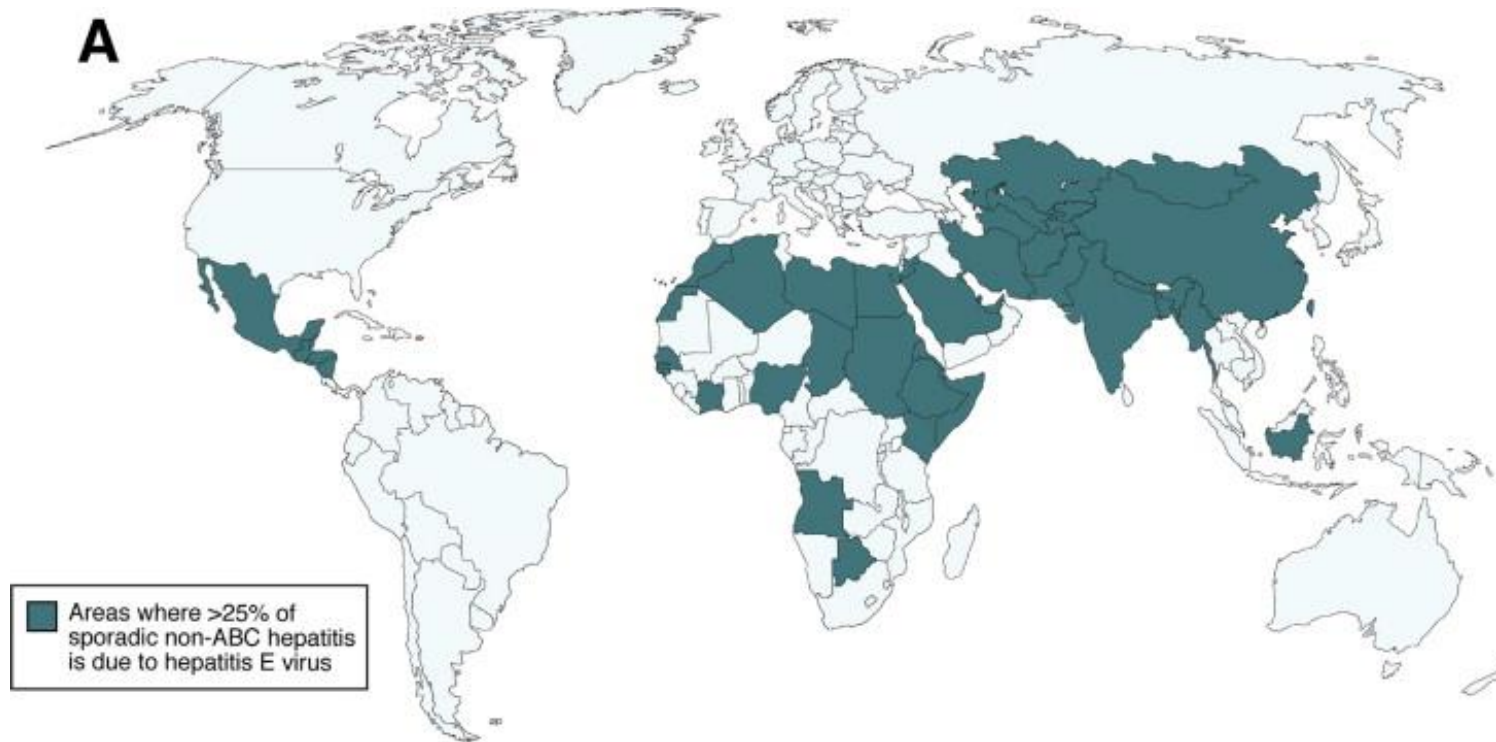
# Under diagnosis (no testing)



**China:** HEV incidence linearly increases (**R<sup>2</sup>=0.82**) in parallel with tests availability

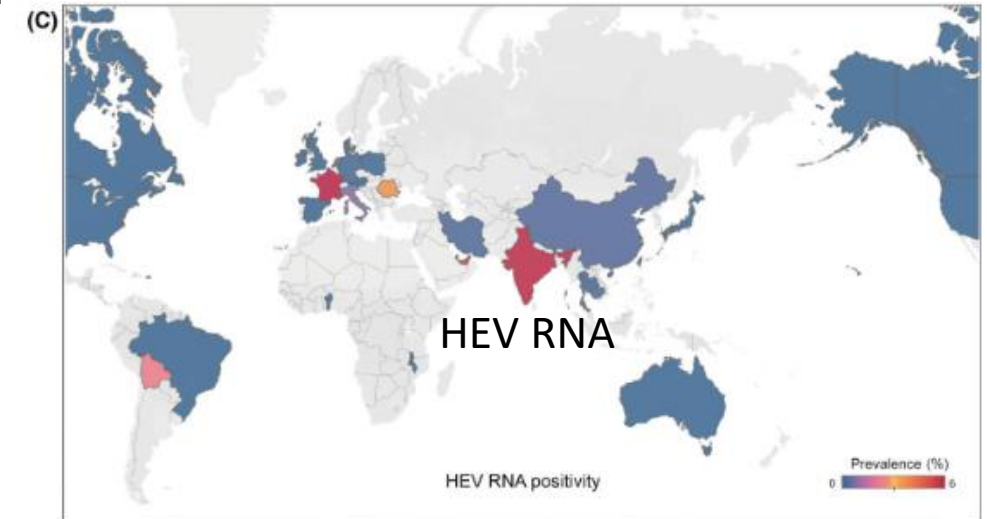
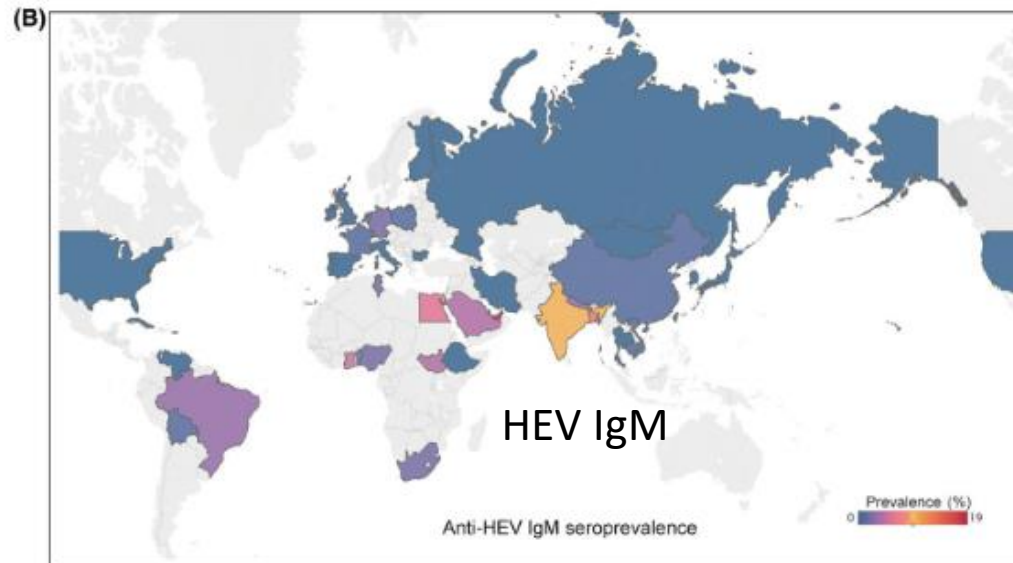
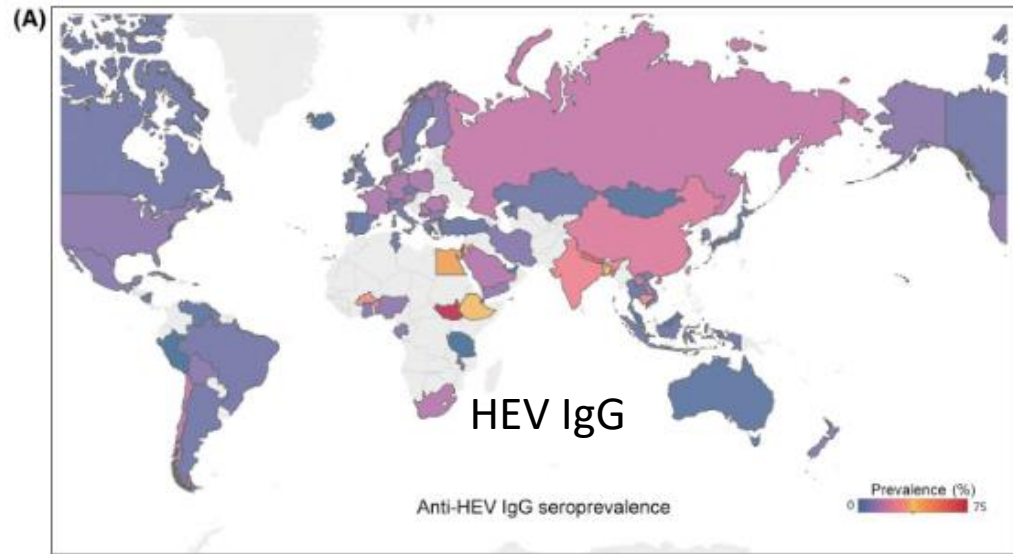
## HEV numbers (WHO estimate):

- Every year 20 million HEV infections, over 3 million acute cases, overall mortality rate range from 0.2% to 4%; i.e. up to 80,000 HEV-related deaths.
- It is the most or second most common cause of acute viral hepatitis among adults throughout much of Asia, the Middle East and Africa.



## Metanalysis: 419 studies up to 2019, 1,519,872 individuals

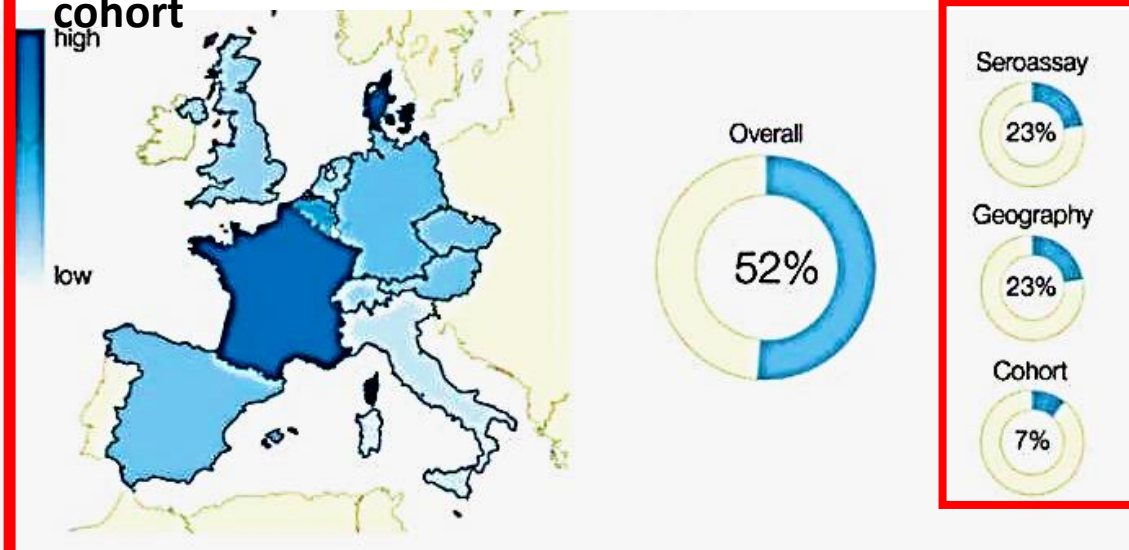
Globally, approximately 939 million corresponding to 1 in 8 individuals have ever experienced HEV infection. 15-110 million individuals have recent or ongoing HEV infection.





73 articles included, 129,254 individuals (Europe)

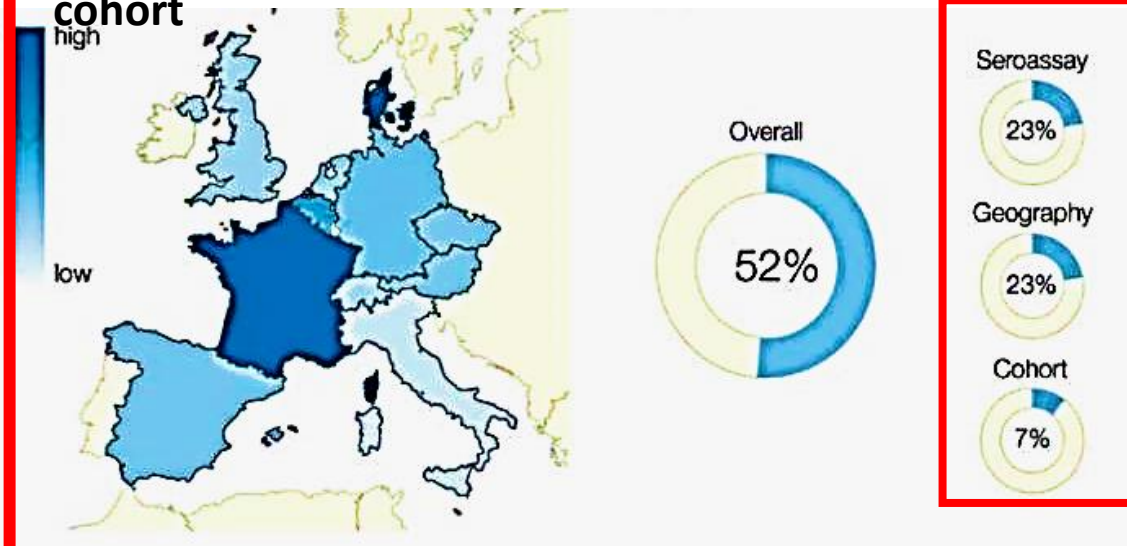
Seroprevalence rates **primarily depend on the seroassay** that is used, followed by the **geographical region** and **study cohort**



Hartl J et al. Viruses. 2016; doi: 10.3390/v8080211

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The detection rates of anti-HEV IgG seropositivity vary dramatically.

Pooled anti-HEV IgG seroprevalence among general population based on different ELISA manufactures

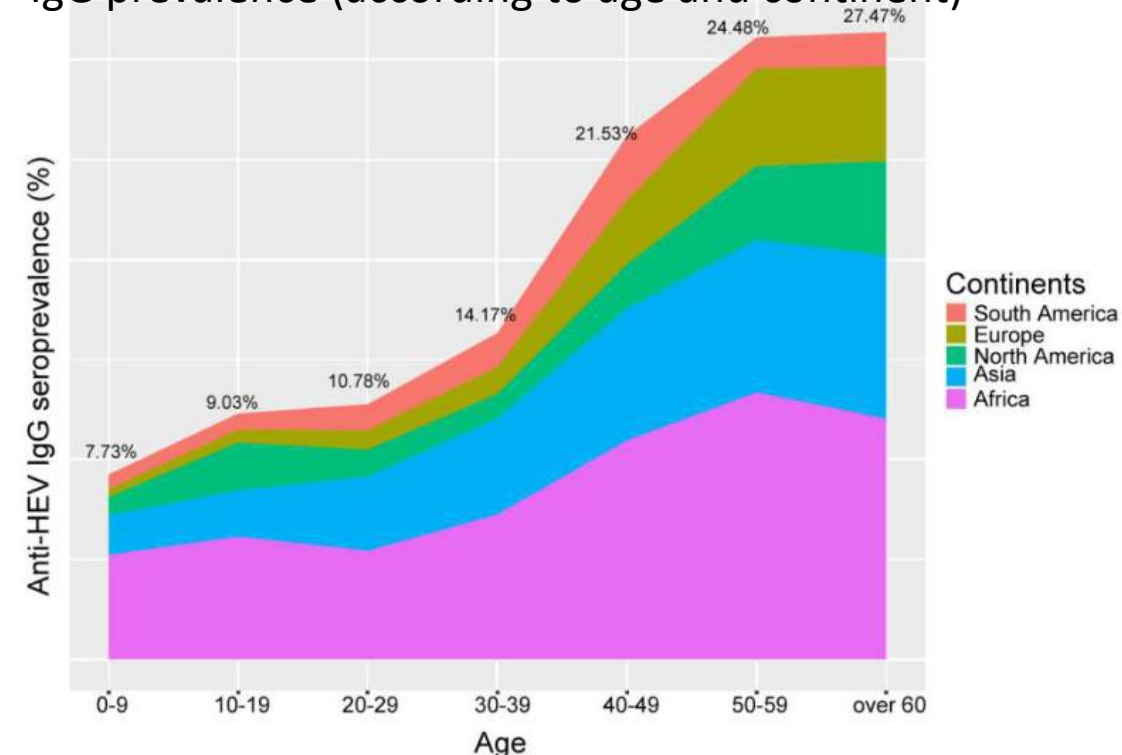
Wantai	20.72%	(95% CI 2.07-9.84)
MP Diagnostics	10.75%	(95% CI 4.55-19.15)
Dia.Pro	8.89%	(95% CI 6.68- 11.38)
Mikrogen	8.60%	(95% CI 4.65-13.62)
Genelabs Diagnostics	6.22%	(95% CI 3.42-9.7)7
Abbott Laboratories	5.27%	(95% CI 2.07-9.84)

419 studies up to 2019, 1,519,872 individuals

**Risk factors for anti-HEV IgG positivity:**

- consumption of raw meat (P = .0001)
- exposure to soil (P < .0001)
- blood transfusion (P = .0138)
- travelling to endemic areas (P = .0244)
- contacting with dogs (P = .0416)
- living in rural areas (P = .0349)
- education >elementary school (P < .0001)

IgG prevalence (according to age and continent)

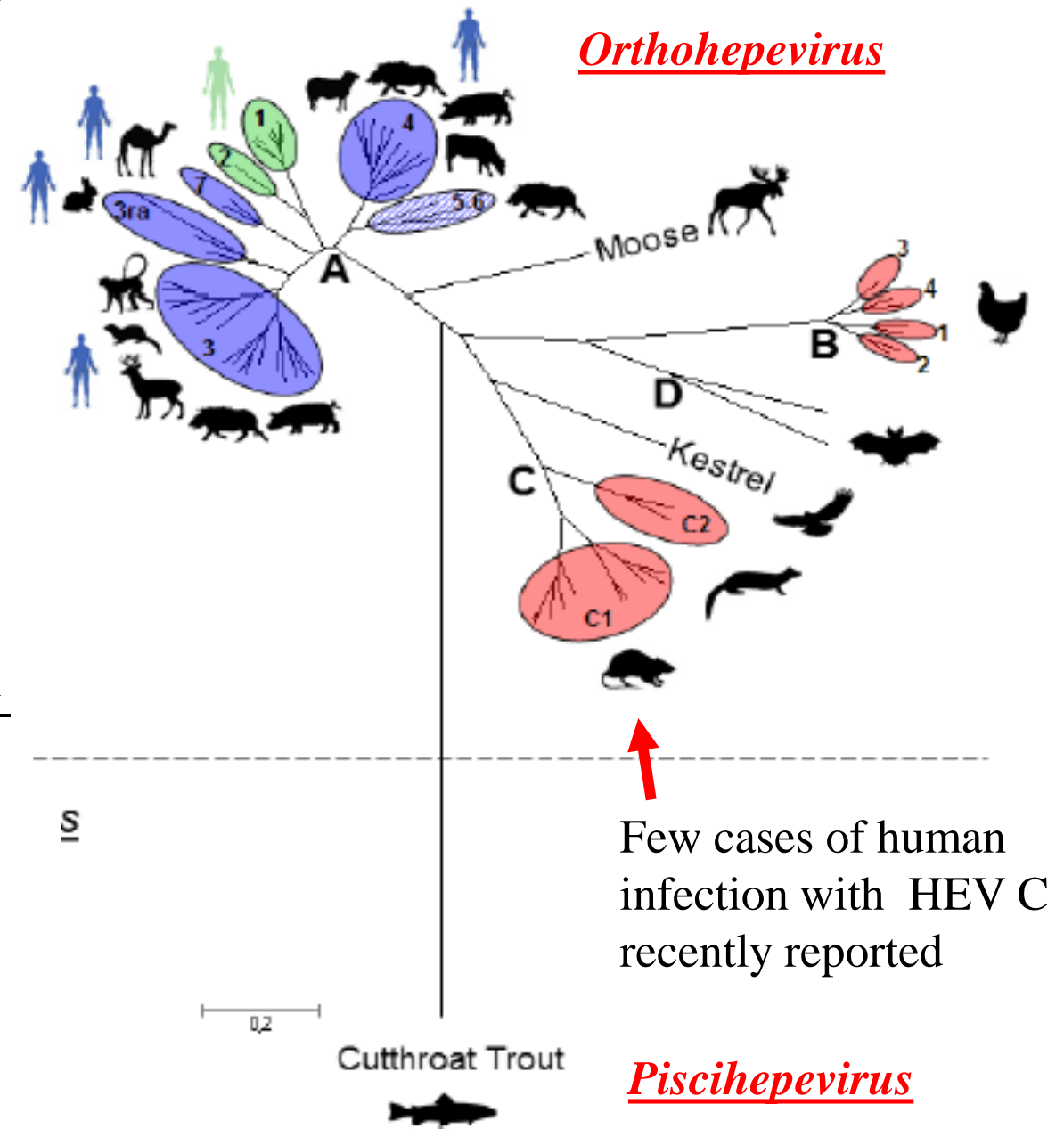


Li P et al. Liver Int. 2020 doi: 10.1111/liv.14468

## Family *Hepeviridae*

Two genera: - *Piscihepevirus*  
- *Orthohepevirus*  
mammalian/avian strains  
**four species (A–D)**

- HEV strains capable of infecting humans were previously thought to be restricted to HEV-1, HEV-2, HEV-3 and HEV-4.
- However, the range expanded following reports of human infection through the regular consumption of camel meat and milk in the United Arab Emirates (HEV-7). HEV-1, HEV-2, HEV-3, HEV-4 and **HEV-7** are all *Orthohepevirus* A.
- The host range has been found to expand to another species—*Orthohepevirus* C [few cases of HEV-C1 (rat) infection recently reported in humans]



*Paslahepevirus*:  
from Primates, Artiodactyla, Scandentia,  
Lagomorpha. the taxa of known hosts of  
mer

*Hepeviridae* Previous and current species names

Previous species

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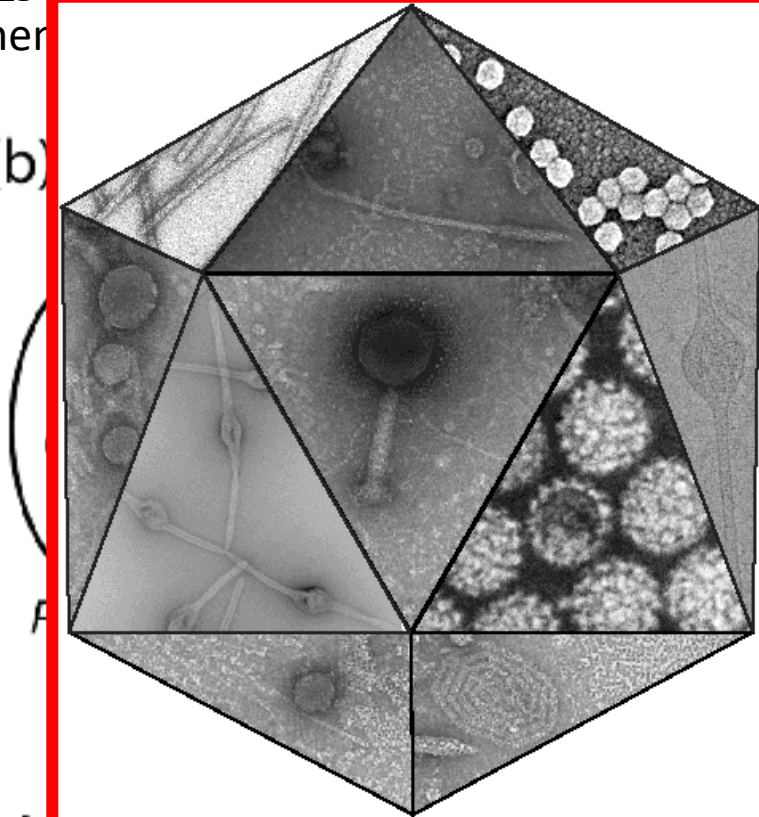
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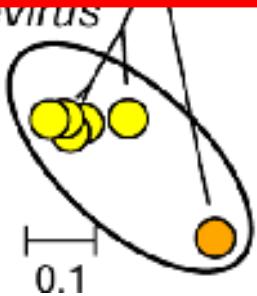
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# Virus Taxonomy

The ICTV Report on  
Virus Classification and Taxon Nomenclature



*Avihepevirus*

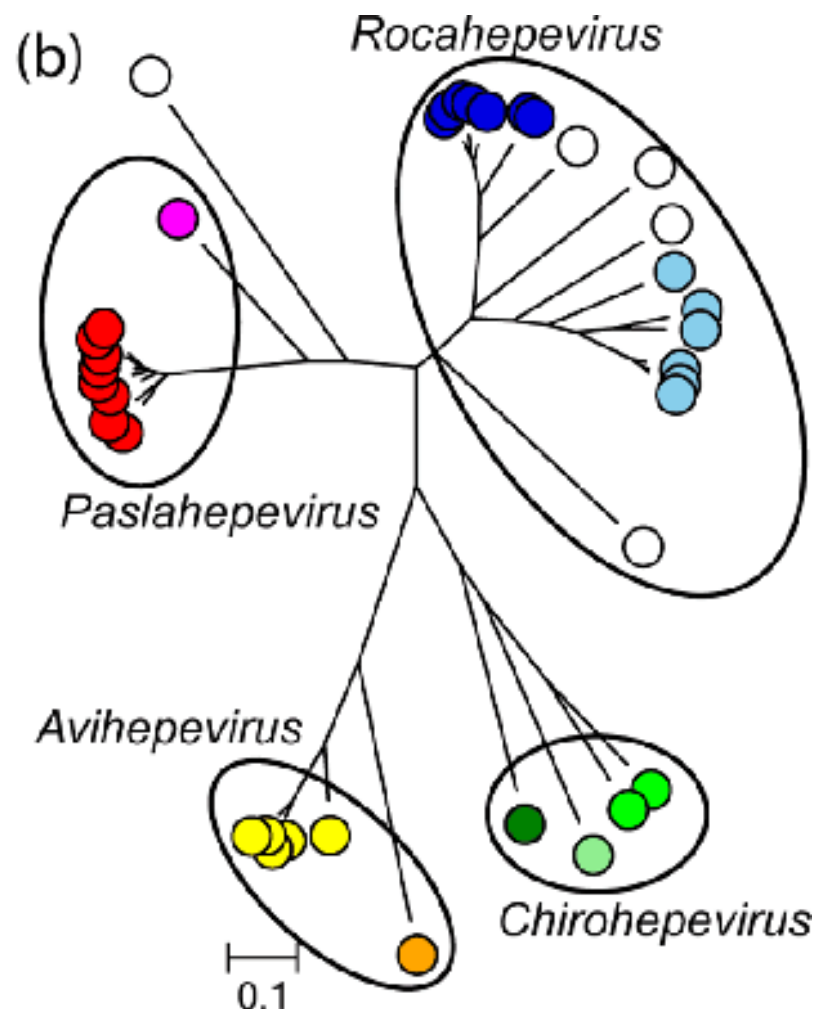


*Chirohepevirus*

Unassigned	HEV of <i>Desmodus</i> bats	<i>Chirohepevirus desmodi</i>
Unassigned	HEV of <i>Rhinolophus</i> bats	<i>Chirohepevirus rhinolophi</i>
<i>Piscihepevirus A</i>	HEV of trout and salmon	<i>Piscihepevirus heenan</i>



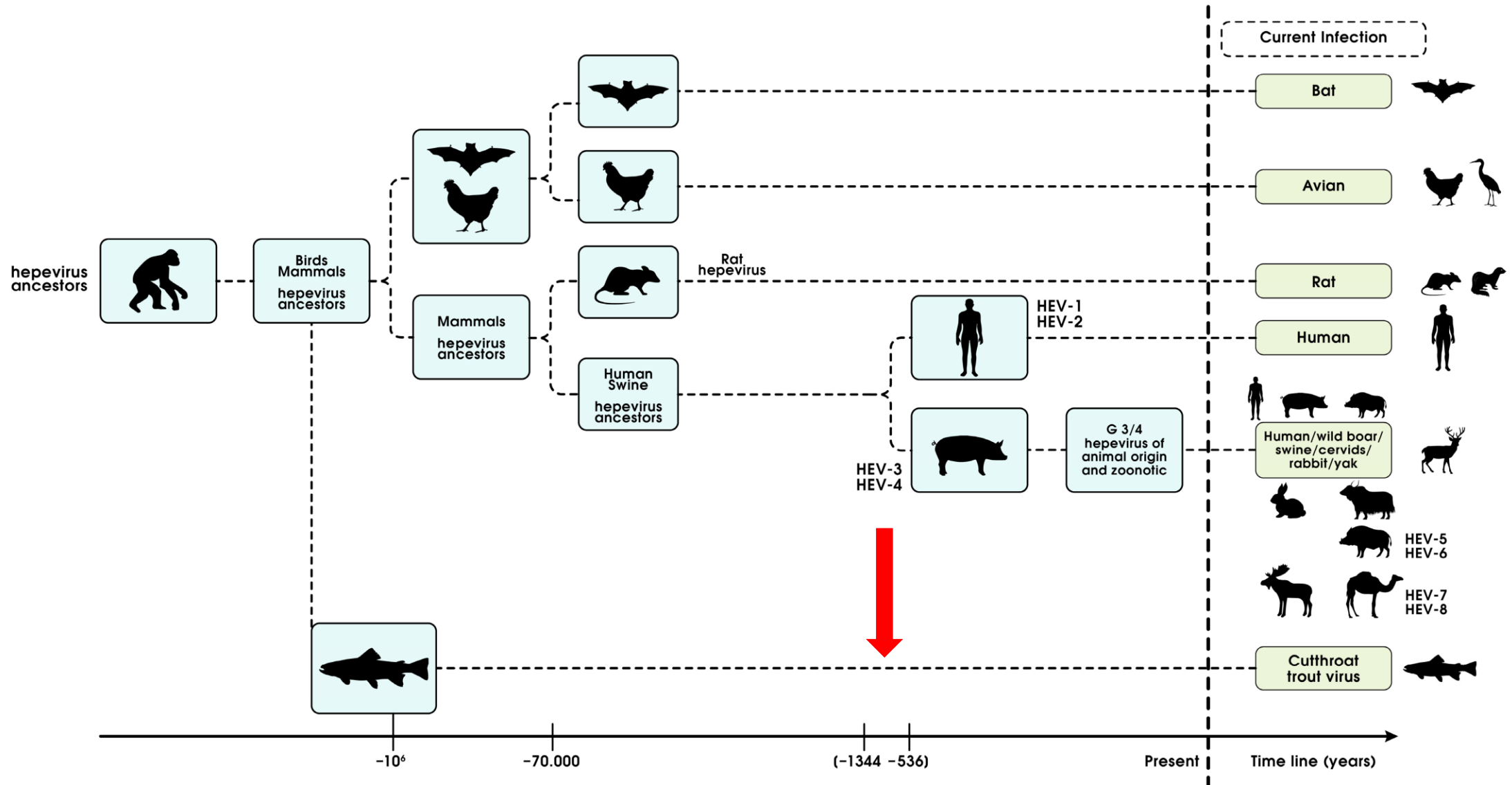
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**Hepeviridae** Previous and current species names

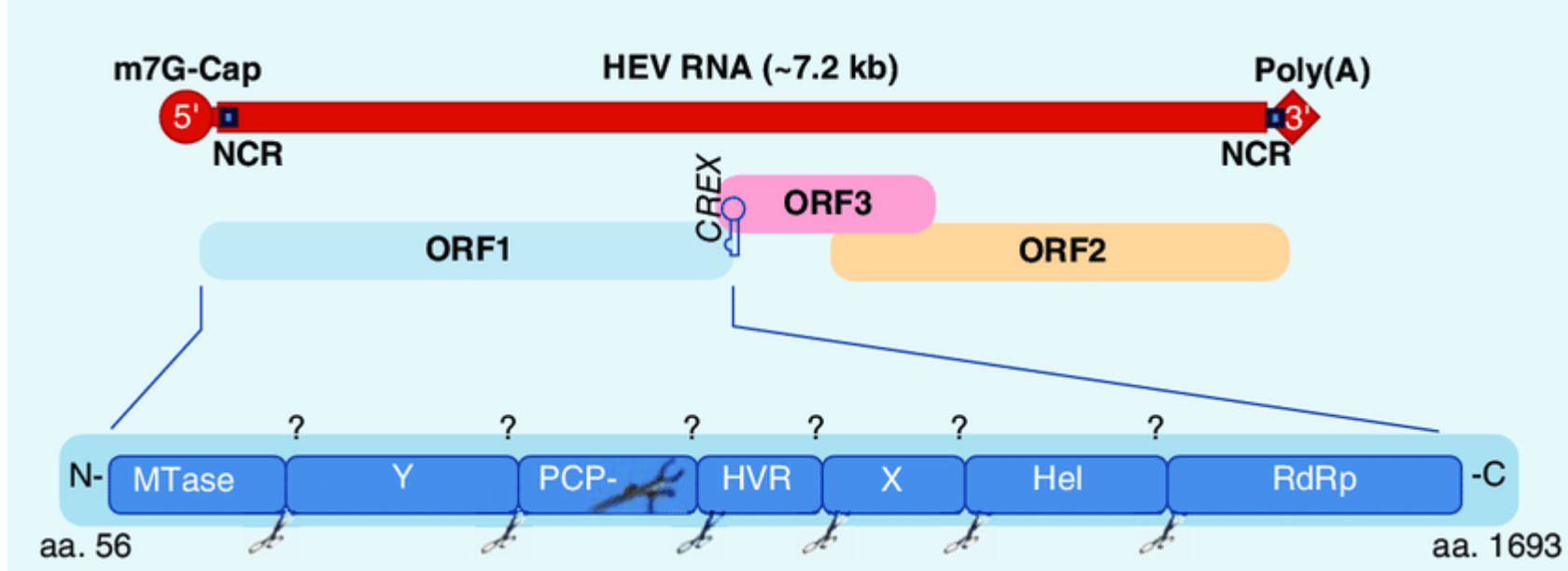
Previous species name	Member viruses	Current species name
<i>Orthohepevirus A</i>	HEV of humans, pigs, deer, wild boar, rabbits, camels etc.	<i>Paslahepevirus balayani</i>
Unassigned	HEV of moose	<i>Paslahepevirus alci</i>
<i>Orthohepevirus B</i>	avian HEV (chickens)	<i>Avihepevirus magniiecur</i>
Unassigned	avian HEV (Little egret)	<i>Avihepevirus egretti</i>
<i>Orthohepevirus C</i>	HEV of rats, ferrets, minks, field mice	<i>Rocahepevirus ratti</i>
Unassigned	HEV of voles	<i>Rocahepevirus eothenomi</i>
<i>Orthohepevirus D</i>	HEV of <i>Eptesicus</i> and <i>Myotis</i> bats	<i>Chirohepevirus eptesici</i>
Unassigned	HEV of <i>Desmodus</i> bats	<i>Chirohepevirus desmodi</i>
Unassigned	HEV of <i>Rhinolophus</i> bats	<i>Chirohepevirus rhinolophi</i>
<i>Piscihepevirus A</i>	HEV of trout and salmon	<i>Piscihepevirus heenan</i>

Around 5 to 13 centuries ago human HEV evolved into anthroponotic HEV-gt1 and 2, affecting only humans, and enzootic HEV-gt3 and 4, affecting pigs and related animals and transmitted to humans through a peculiar food-borne pathway.



# Outline

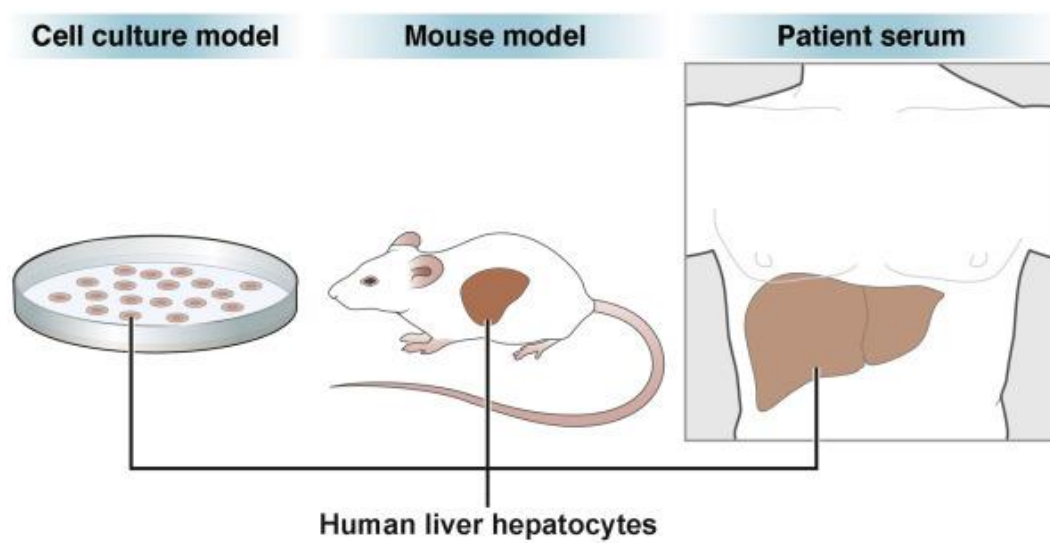
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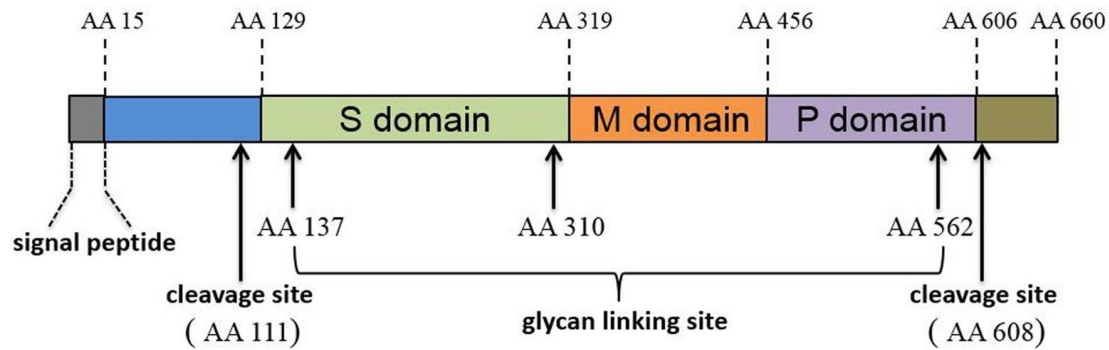
## HEV genome

- 3 partially overlapped open reading frames (**ORF1, ORF2 and ORF3**), including the 5' m7Gcap, 3' poly(A) tail and 5'/3' noncoding regions (NCR).
- **ORF1 encodes for a nonstructural polyprotein** composed of: MTase (methyltransferase), Y (undefined), PCP (papain-like cysteine protease), HVR (hypervariable region), X, Hel (helicase) and RdRp (RNA dependent RNA polymerase)
- **ORF2** encodes for the **viral capsid protein**, and is involved in virion assembly (interaction with RNA)
- **ORF3** encodes for a functional ion channel (**viroporin**) required for the release of infectious virions from host cells, through the ESCRT pathway
- Recently, a novel **ORF4** was predicted from **HEV genotype 1** sequences embedded entirely within ORF1. The role of the ORF4-encoded protein is yet to be clarified





Using HEV infection models in vitro (human hepatoma cells), in vivo (urokinase plasminogen activator transgenic–SCID mice xenografted with primary human hepatocytes) and analyzing patient’s serum  
**3 forms of ORF2 protein were identified:**



Using HEV infection models in vitro (human hepatoma cells), in vivo (urokinase plasminogen activator transgenic–SCID mice xenografted with primary human hepatocytes) and analyzing patient’s serum **3 forms of ORF2 protein were identified:**



- ORF2<sub>i</sub>, associated with infectious virus particles
- ORF2<sub>g</sub> full-length glycosylated form
- ORF2<sub>c</sub> truncated glycosylated form

Hepatitis E virus



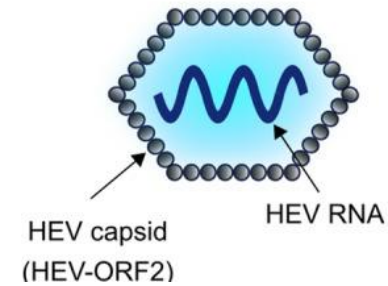
Infectious particle ORF2i



- The identification of both conformational and linear neutralizing epitopes in ORF2 suggests that it is the target for neutralizing antibodies
- The secretion of distinct forms of the capsid protein that are not incorporated into infectious virions is reminiscent of the secretion of HBeAg in HBV infection and suggests an immunomodulatory role and an immune escape mechanism

Generally, a viral envelope is formed by a budding process from membranes of infected cells, with at least one virus-encoded glycoprotein (peplomer) embedded in this layer

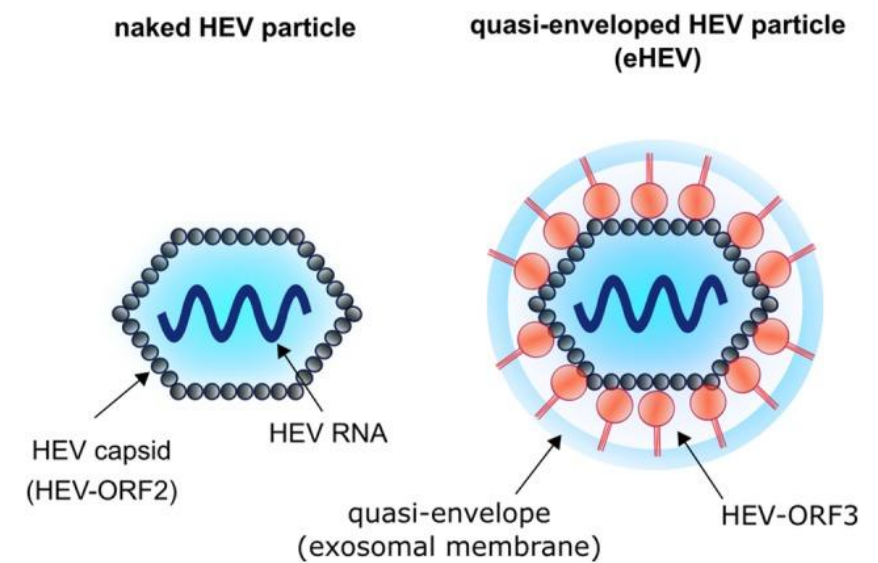
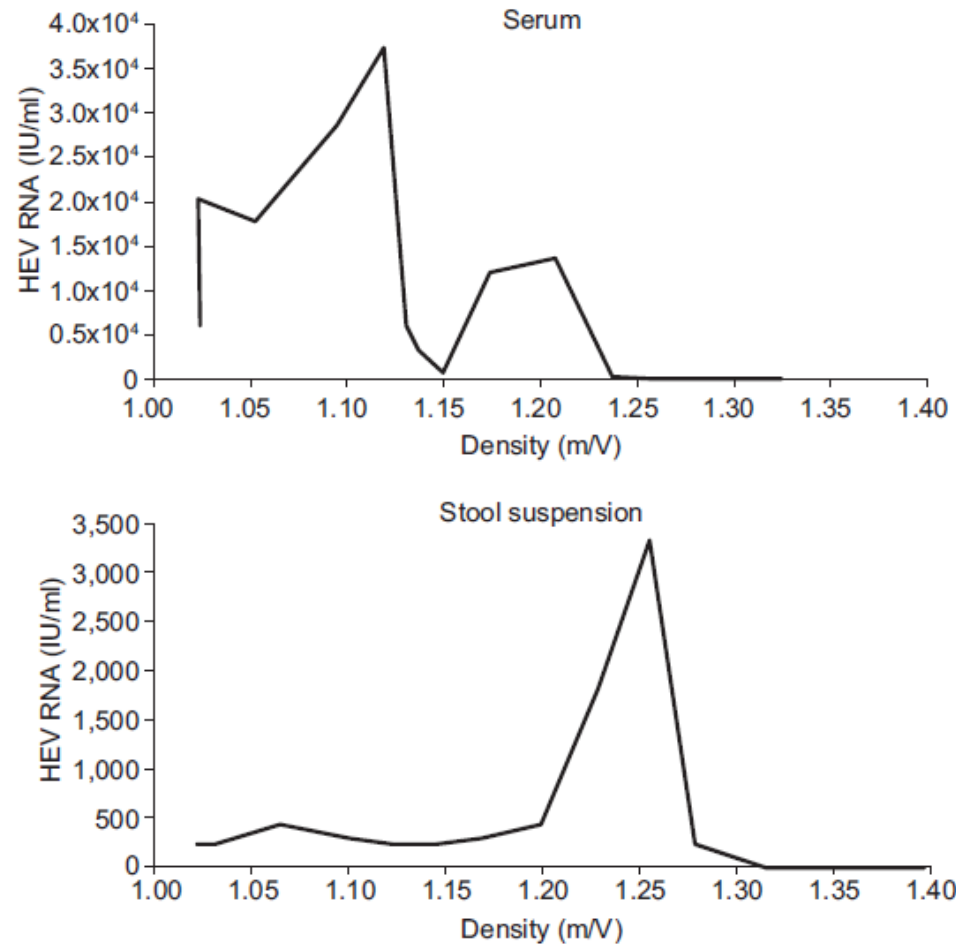
Viral proteins embedded in the envelope mediate diverse interactions with cellular receptors



- HEV was thought to be non-enveloped for decades
- Recent studies have revealed that the virus circulating in the patient's blood is completely cloaked in host membranes and resistant to neutralizing antibodies
- The HEV particles present in feces and bile are non-enveloped, while those in circulating blood and culture supernatants are covered with a cellular membrane, similar to enveloped viruses

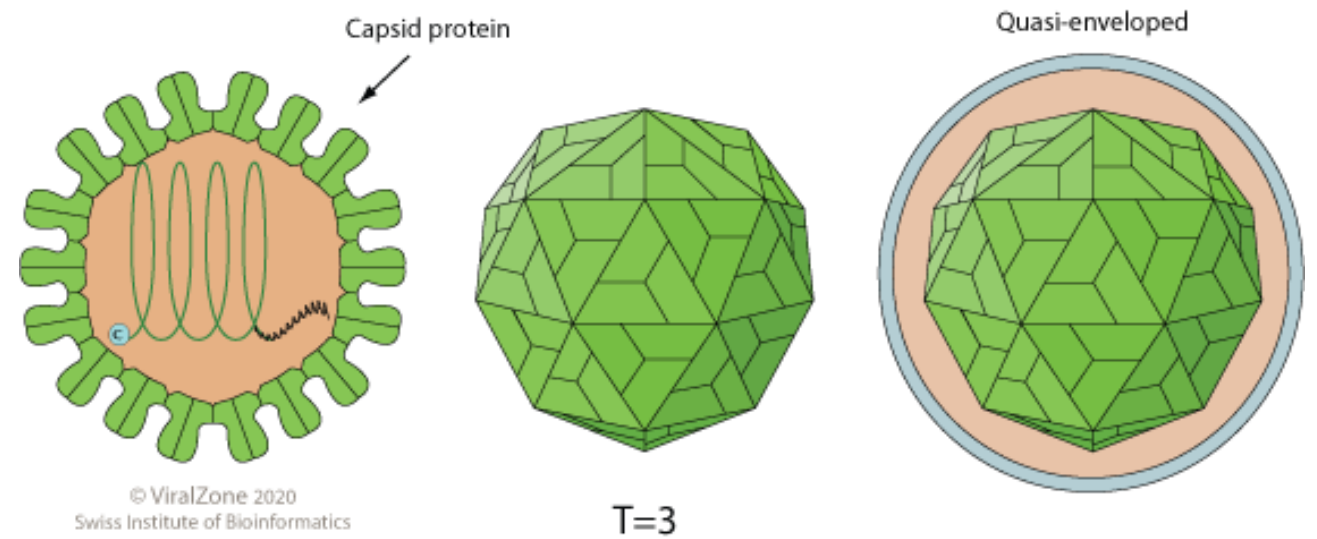
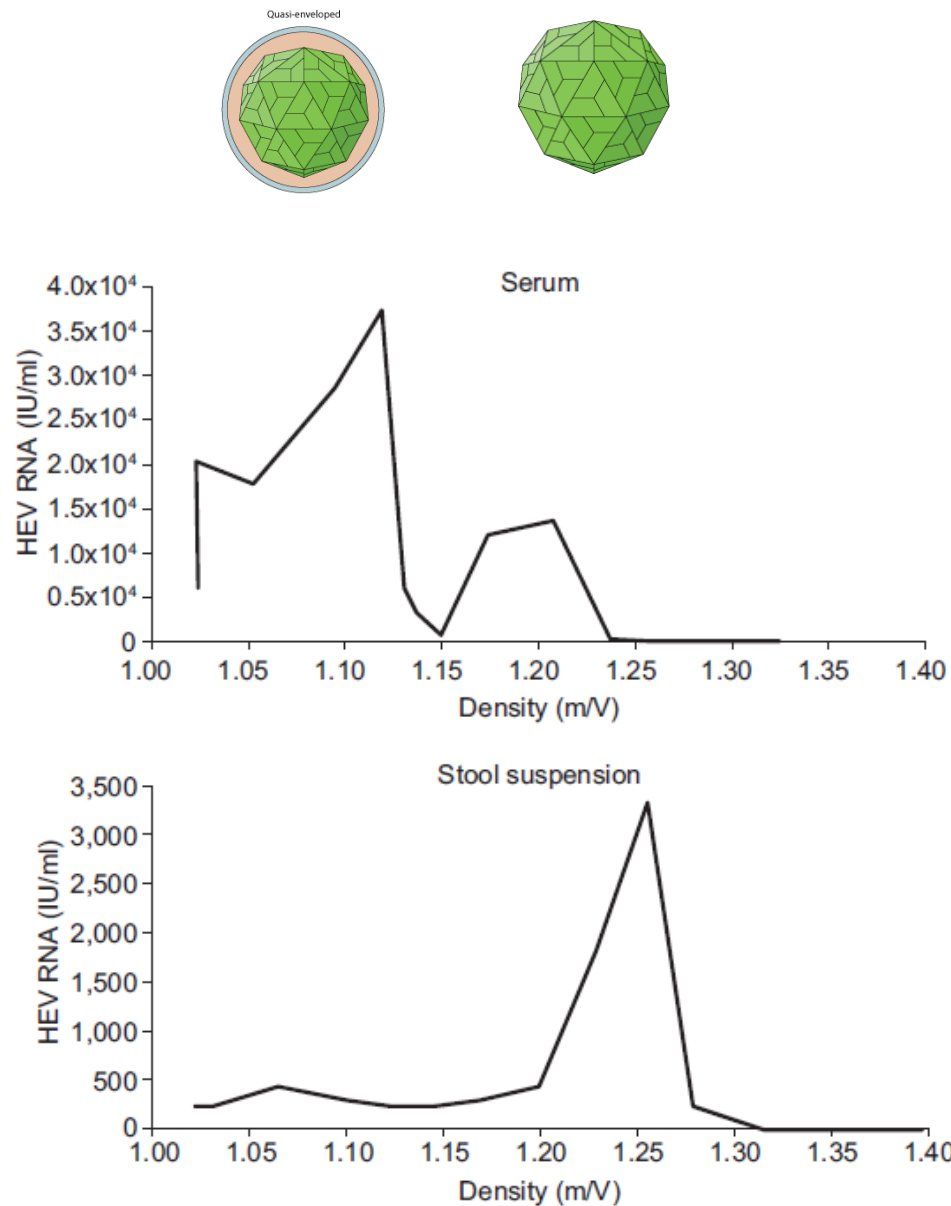
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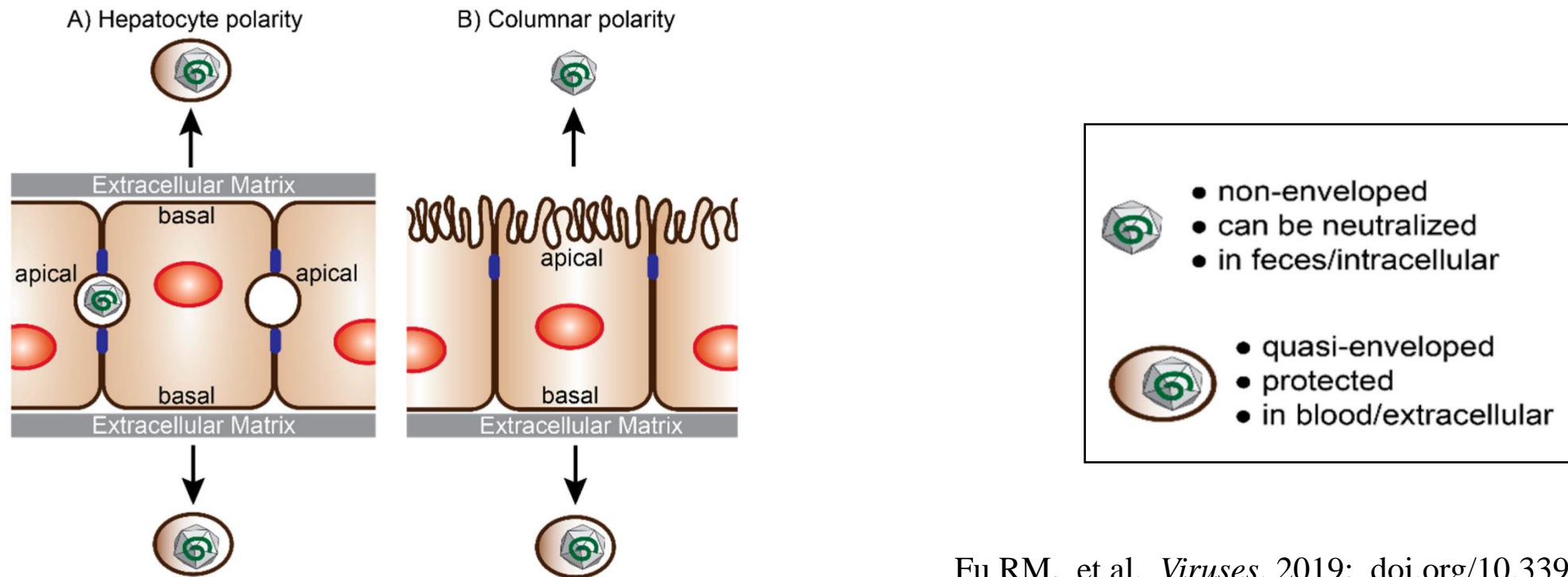
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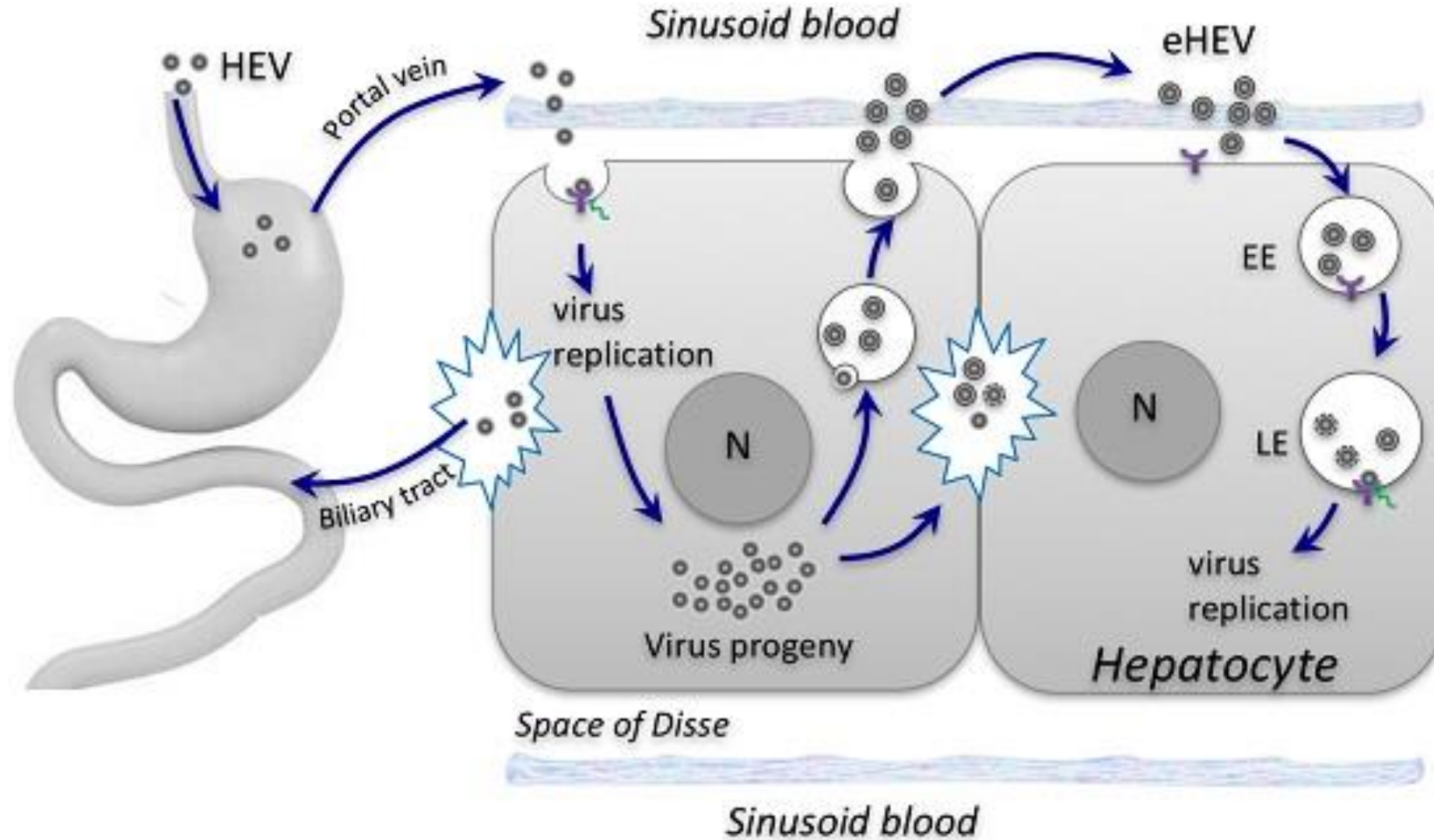


- Quasi-enveloped, spherical, about 32-34 nm in diameter.
- 180 capsid proteins, assembled into a T=3 icosahedral particle in an RNA-dependent manner.
- The HEV particles present **in feces and bile** are **non-enveloped**, while those in circulating **blood and culture supernatants** are covered with a cellular membrane, similar to **enveloped** viruses

- Likely, HEV first replicates in the intestinal tract before reaching the liver via the blood in a quasi-enveloped form
- In an in vitro model of polarized hepatocytes HEV particles are released at the apical membrane, that is, the bile side.
- Infected hepatocytes release HEV as lipid-associated particles into the blood and bile. Bile salts then strip the lipids from the virus shed in the stool



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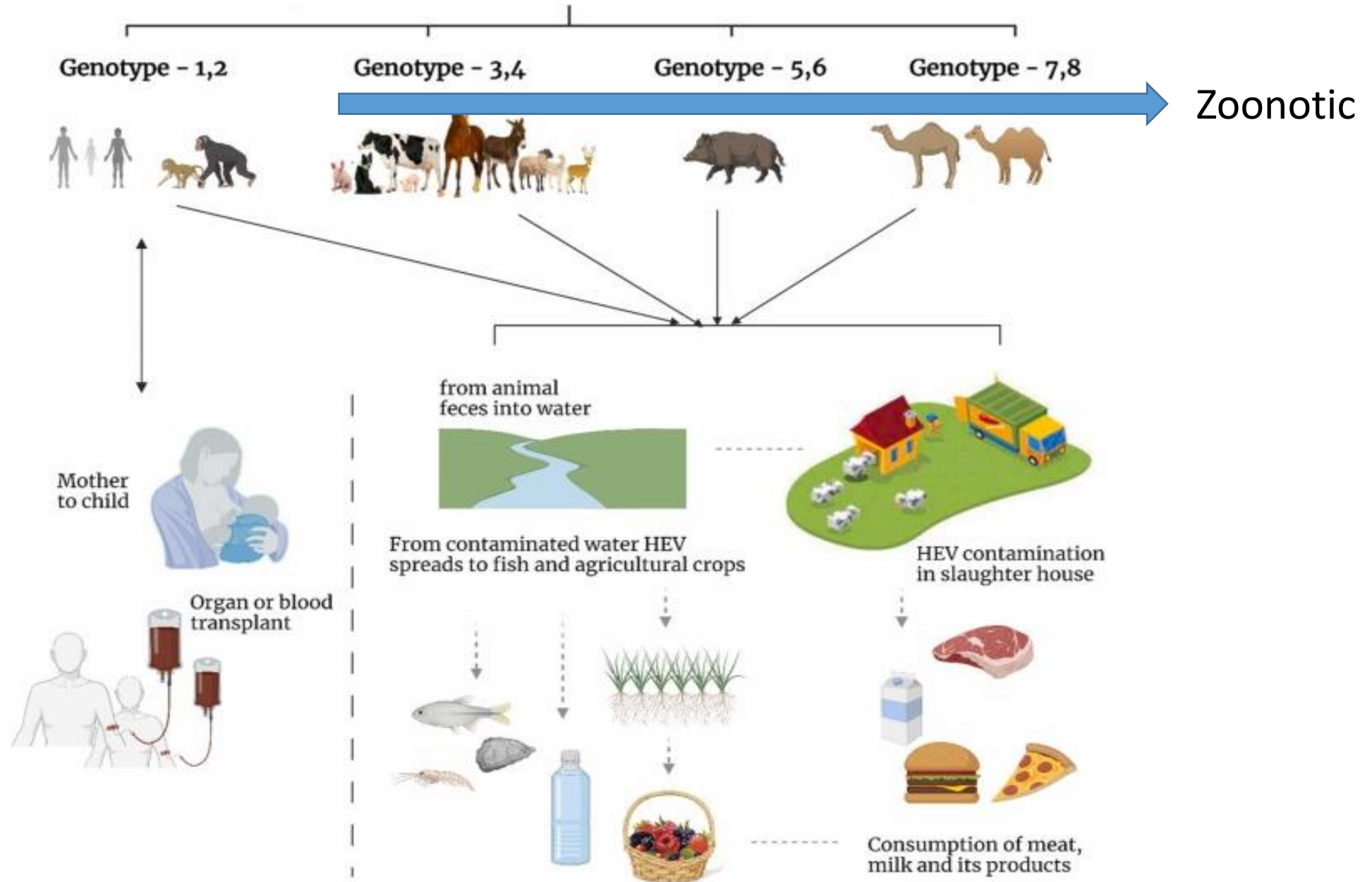


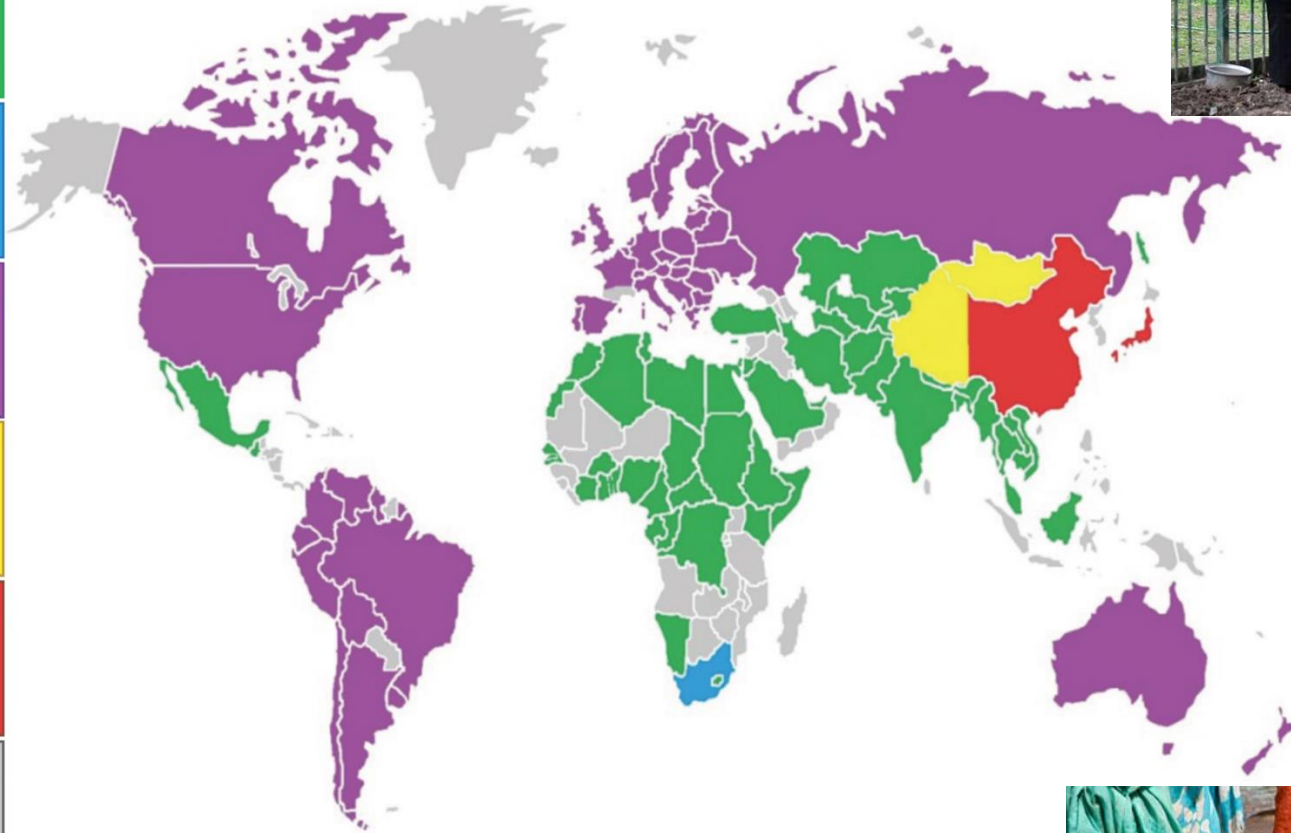
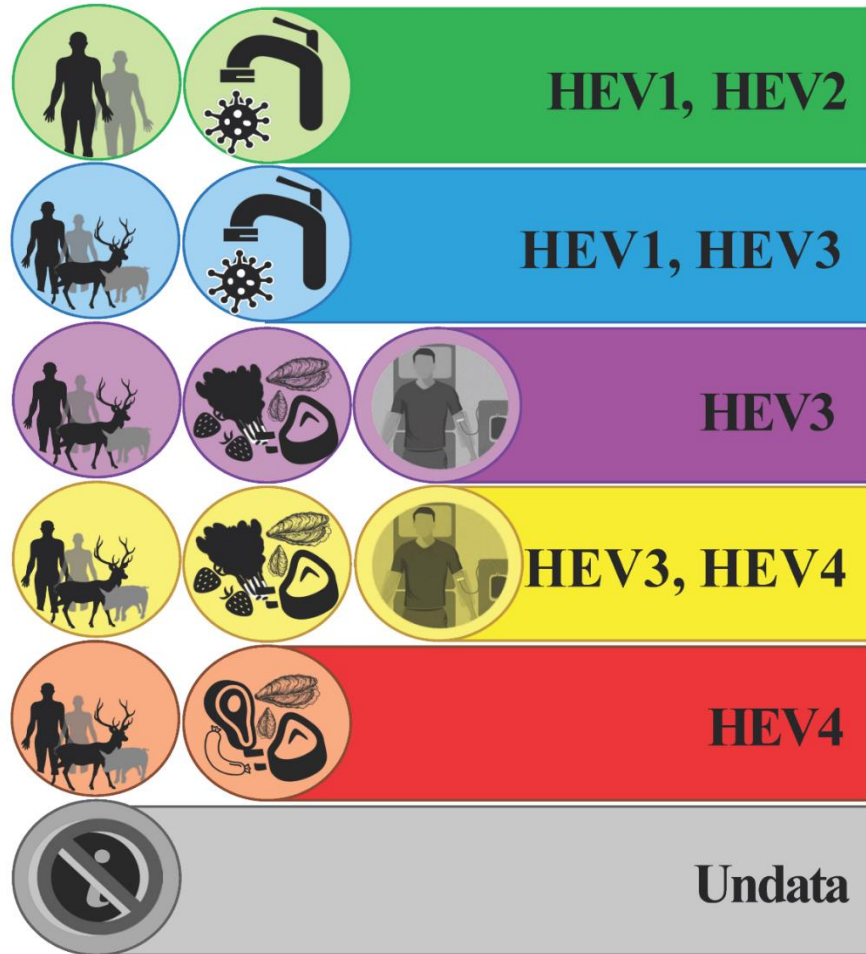
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# HEV transmission routes





Gen 3-4



Gen 1-2



## Developing countries

- HEV1-HEV2
- Epidemic (water borne?), young adult males (15–30 years)
  - ✓ asymptomatic,
  - ✓ mild systemic illness,
  - ✓ icteric acute hepatitis that can be fulminant or lead to acute liver failure.
- High deaths in pregnancy
  - ✓ in second and third trimester of **pregnancy** can progress to acute liver failure(25%)
- Extra-hepatic disease
- **Chronic infection not reported**

## Developed countries

- HEV3-HEV4
- Sporadic, autochthonous, small clusters
- Middle-aged or elderly men
- Occurrence of symptoms possibly linked to viral load
- Severe infection in **pregnancy not reported**
- Extra-hepatic disease
- **Chronic infection** (HEV-3, in immunocompromised: SOT, HIV, etc.)

## Annual incidence estimates:

0.2% in UK (Ijaz S, J Clin Virol 2009)

0.7% in US (Faramawi MF, Epidemiol Infect 2011)

4.3% in China (Li RC, Emerg Infect Dis 2006)

2.1% in South France (Abravanel F, JID 2014)

other

## **Hepatitis E Virus In Italy: Molecular Analysis Of Travel-Related And Autochthonous Cases**

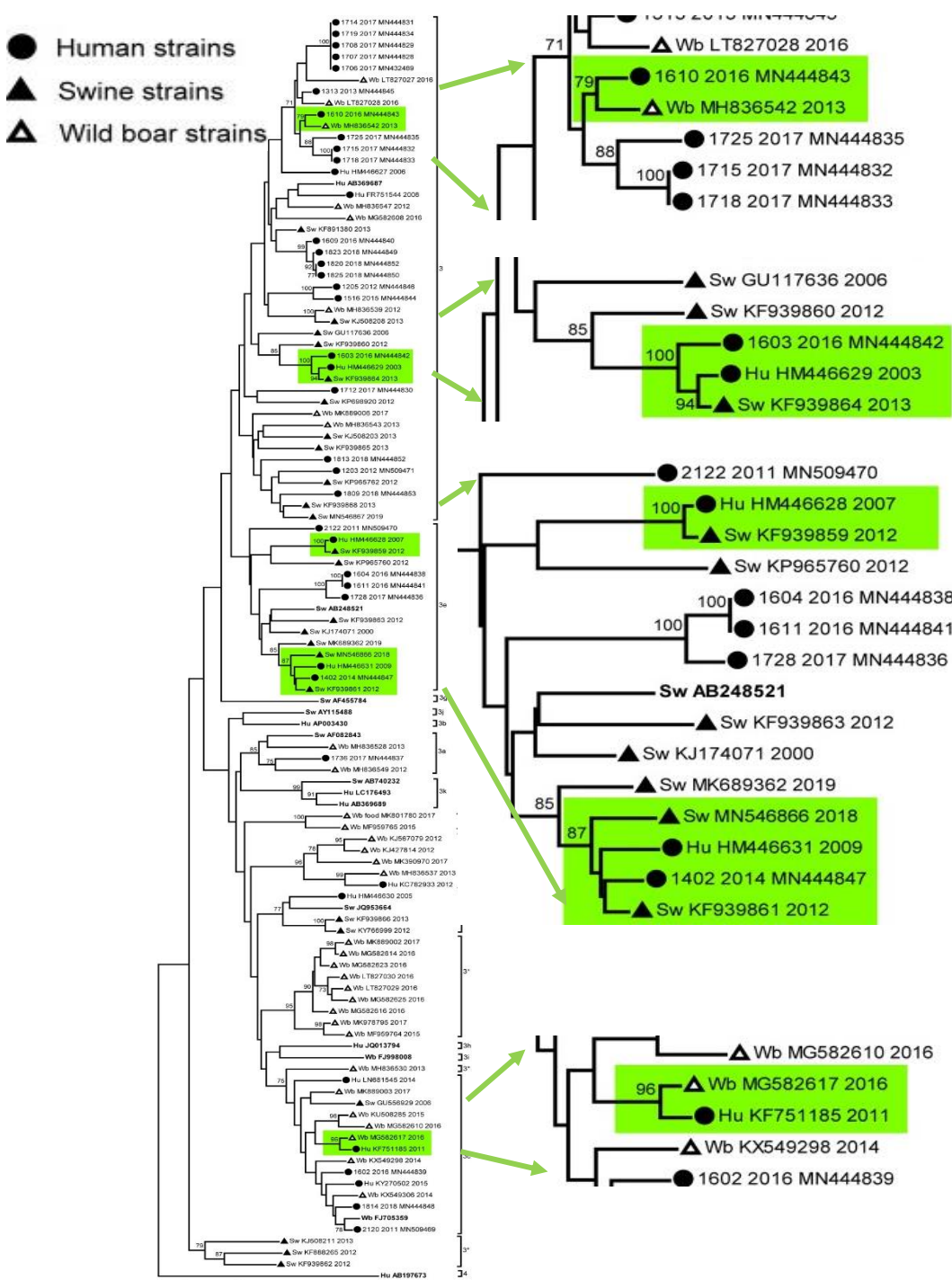
**Giuseppina La Rosa<sup>1,3</sup>, Michele Muscillo<sup>1</sup>, Valentina Spuri Vennarucci<sup>1</sup>,  
Anna Rosa Garbuglia<sup>2</sup>, Patrizia La Scala<sup>2</sup> and Maria Rosaria Capobianchi<sup>2</sup>**

<sup>1</sup> Istituto Superiore Sanita, Roma;

<sup>2</sup> National Institute for Infectious Diseases 'L. Spallanzani', Rome, Italy

Human HEV infection in Italy is caused by different genotypes, depending on whether the infection is **travel-related (gen 1)** or **autochthonous (gen 3)**.





# Molecular Characterization of HEV Genotype 3 in Italy at Human/Animal Interface

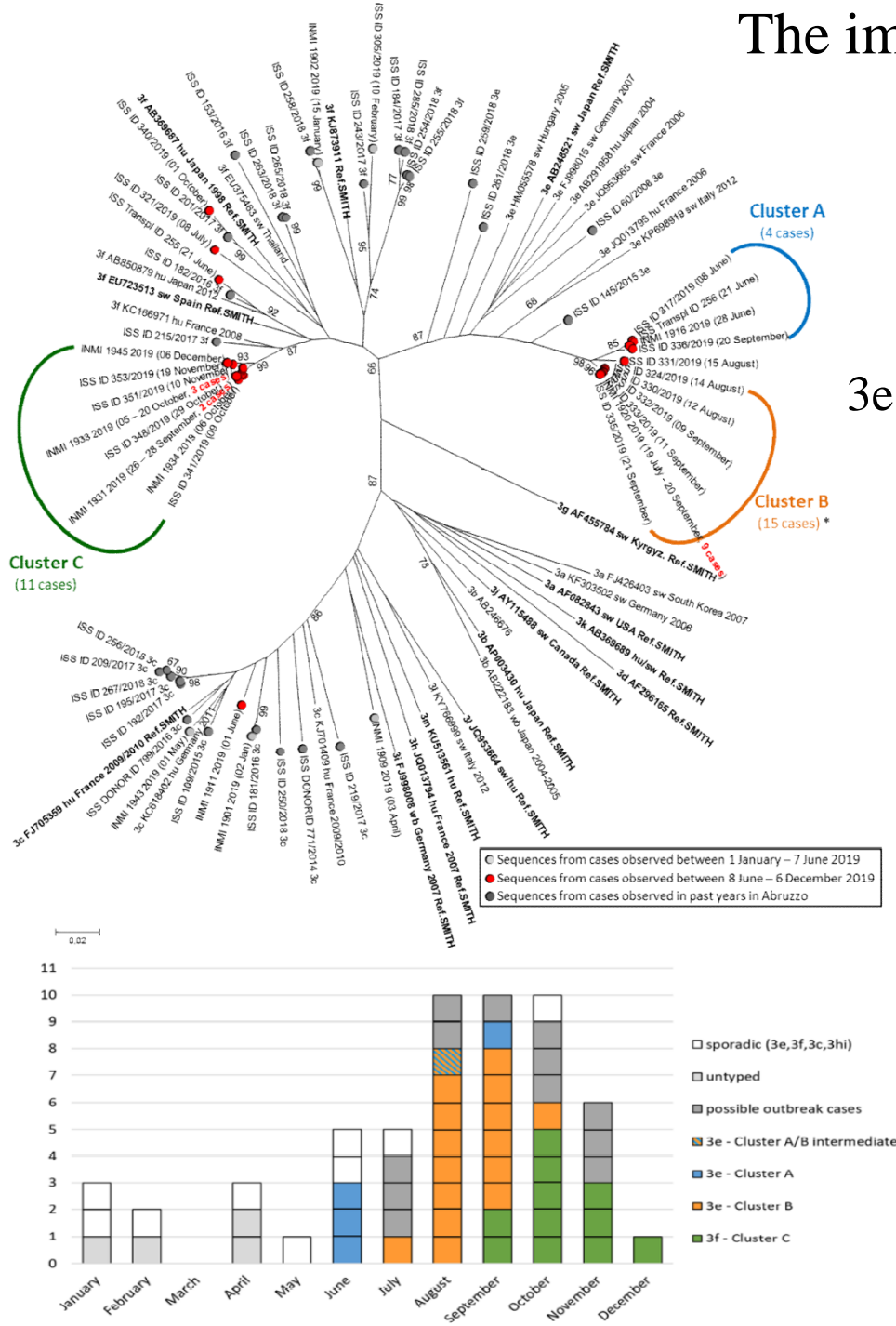
Luca De Sabato<sup>1†</sup>, Ilaria Di Bartolo<sup>1†</sup>, Daniele Lapa<sup>2</sup>, Maria Rosaria Capobianchi<sup>2\*</sup> and Anna Rosa Garbuglia<sup>2</sup>

- Subtype distribution of HEV-3 ( $n = 96$ ) in humans and animals (swine and wild boar) from Italy (2000-2018)
- Human and animal sequences forming related clusters are highlighted in green
- 3f is the most frequent in humans and animals, followed by the HEV-3e, HEV-3c
- HEV-3c human sequences from Italy, rarely detected in Italian pigs, are more correlated to human sequences from other European countries



# The importance of molecular surveillance

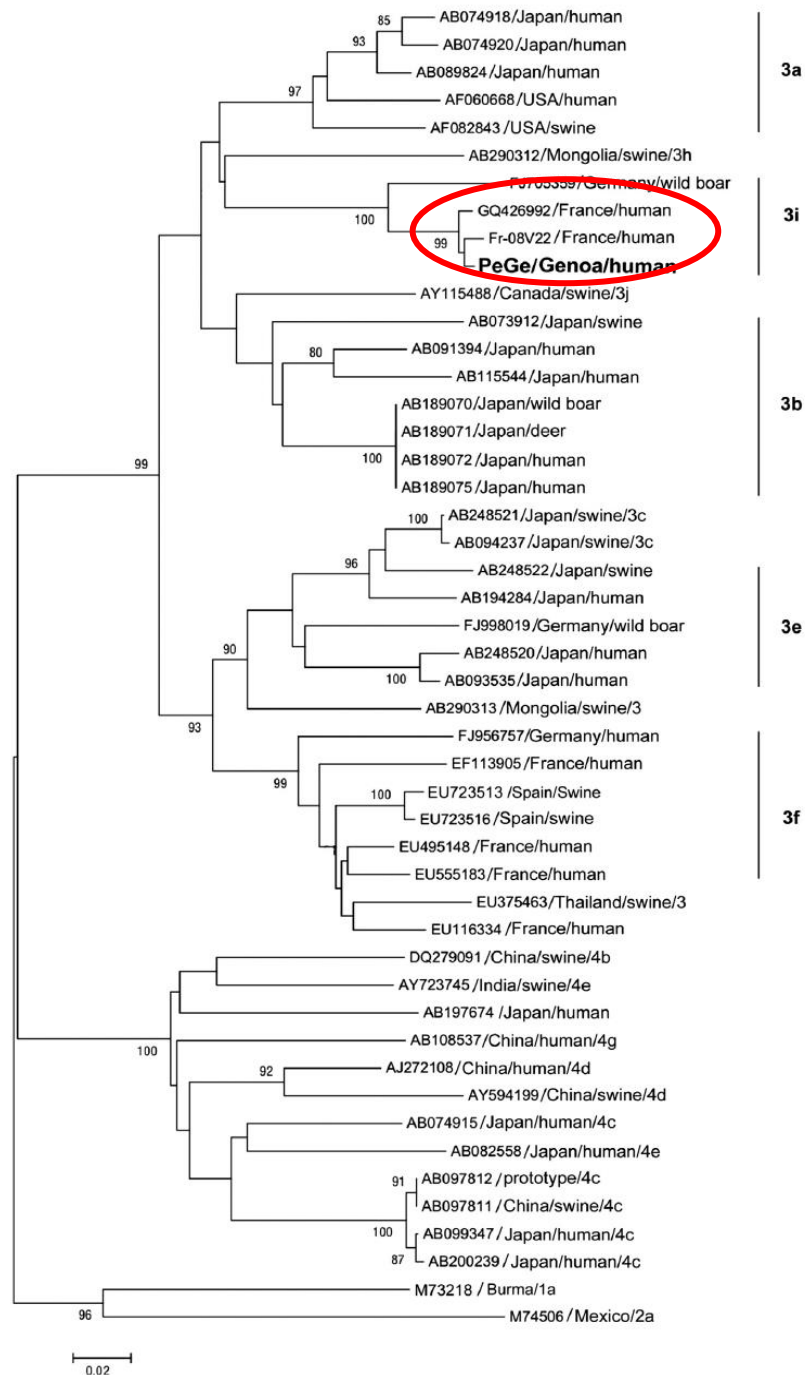
3f



3e

- In mid/late September 2019, some **identical/highly similar HEVgt3 sequences** observed at Virology Lab (INMI, Rome) prompted a common effort with ISS to investigate possible outbreak
- An **outbreak** was identified (47 cases from 8 June 2019 to 6 December 2019), involving Abruzzo and Lazio
  - ✓ Three asymptomatic HEV RNA positive infections were from SOT individuals attending the Regional Transplant Centre of Abruzzo and Molise
  - ✓ Most Lazio cases linked with Abruzzo
- Phylogenetic analysis of the HEV showed **molecular clusters** (A, B, and C)
  - ✓ A and B clusters subtype 3e
  - ✓ C cluster subtype 3f
- Partially overlapped temporal distribution of the three clusters
- Pork products most likely source of the outbreak, although food investigation did not show HEV in local pork products
- No similarity with previous strains in Abruzzo, but **high similarity with strains circulating in East Europe**
- Almost simultaneously introduced new strains possibly linked to pork meat importation

# The first case of HEV genotype 3 infection imported from an industrialized country



3a

3i

3b

3e

3f

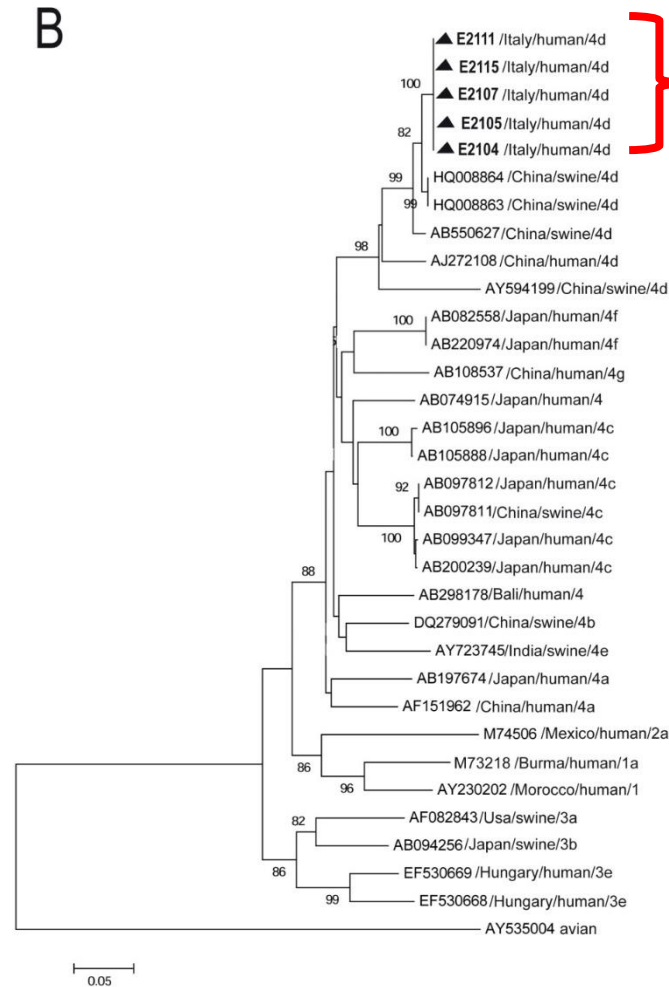


**Male patient with acute hepatitis E in Genoa, Italy: figatelli (pork liver sausage) as probable source of the infection**

A. R. Garbuglia<sup>1</sup>, A. I. Alessandrini<sup>2</sup>, N. Pavio<sup>3</sup>, S. Tessé<sup>4</sup>, S. Grignolo<sup>2</sup>, C. Viscoli<sup>5</sup>, D. Lapa<sup>1</sup> and M. R. Capobianchi<sup>1</sup>

- A 44-year-old man from Genoa took his vacation in Corsica and in Southern France (late August-early of September 2011).
- During his holiday, he bought figatelli in **Corsica** and stored it at +4°C until September 21, 2011, when he ate uncooked.
- After about 40 days he developed hepatitis.
- HEV gt 3i (first time in Italy) was diagnosed.
- Consumption of figatelli was the only identified factor.
- ORF-2 sequence was most closely related only to 2 HEV 3i strains circulating in the area of **Southern France** where figatelli had been purchased

Between March and April 2011, acute hepatitis E was diagnosed in five patients admitted to three Hospitals in Lazio Region  
 The five patients lived at a maximum distance of 27 kilometres



- The high genetic similarity among the Italian HEV gt4 strains supports **point-source outbreak**, and argues against local circulation
- Epidemiological information did not allow to identify the transmission route, although
  - The available data allow to rule out direct inter human transmission
  - Transmission via parenteral route is unlikely (no history of blood transfusion, tattooing, IV drug use)
- The highest genetic similarity of Italian isolates with genotype 4d strains of swine origin from **China** supports possible origin of the outbreak through **imported pork or meat**

# Hepatitis E-A virus with two faces

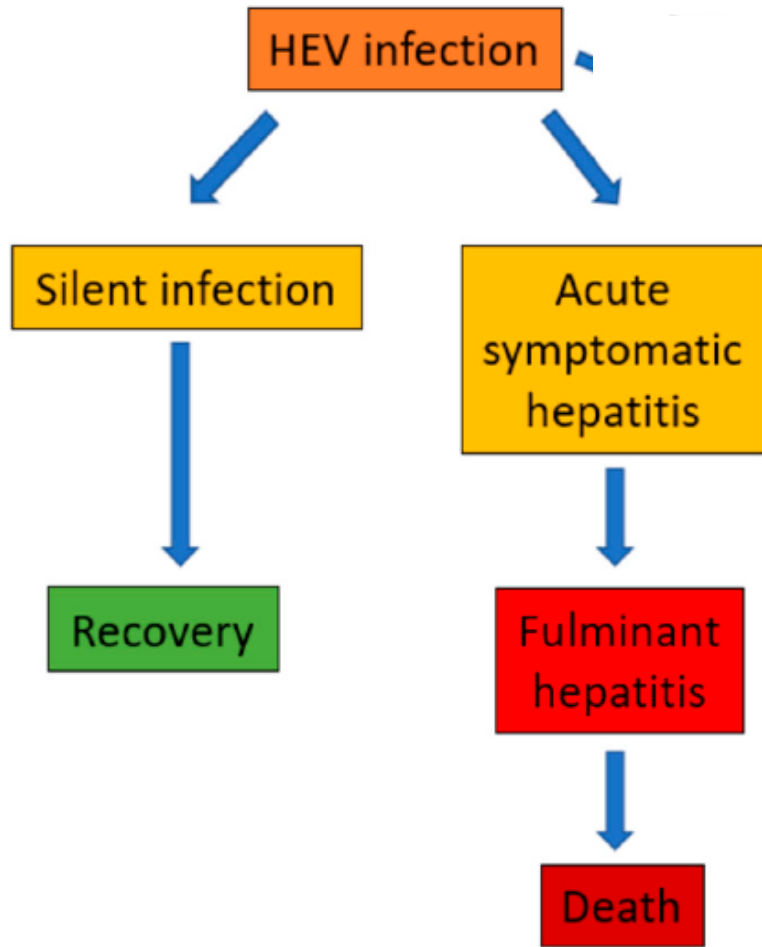


The effects of HEV are usually self-limiting, but in rare cases it can manifest into acute liver failure.

- Typically, pregnant women are at greatest risk of dying from this liver failure
- People with weak immune systems are more likely to suffer from chronic cases

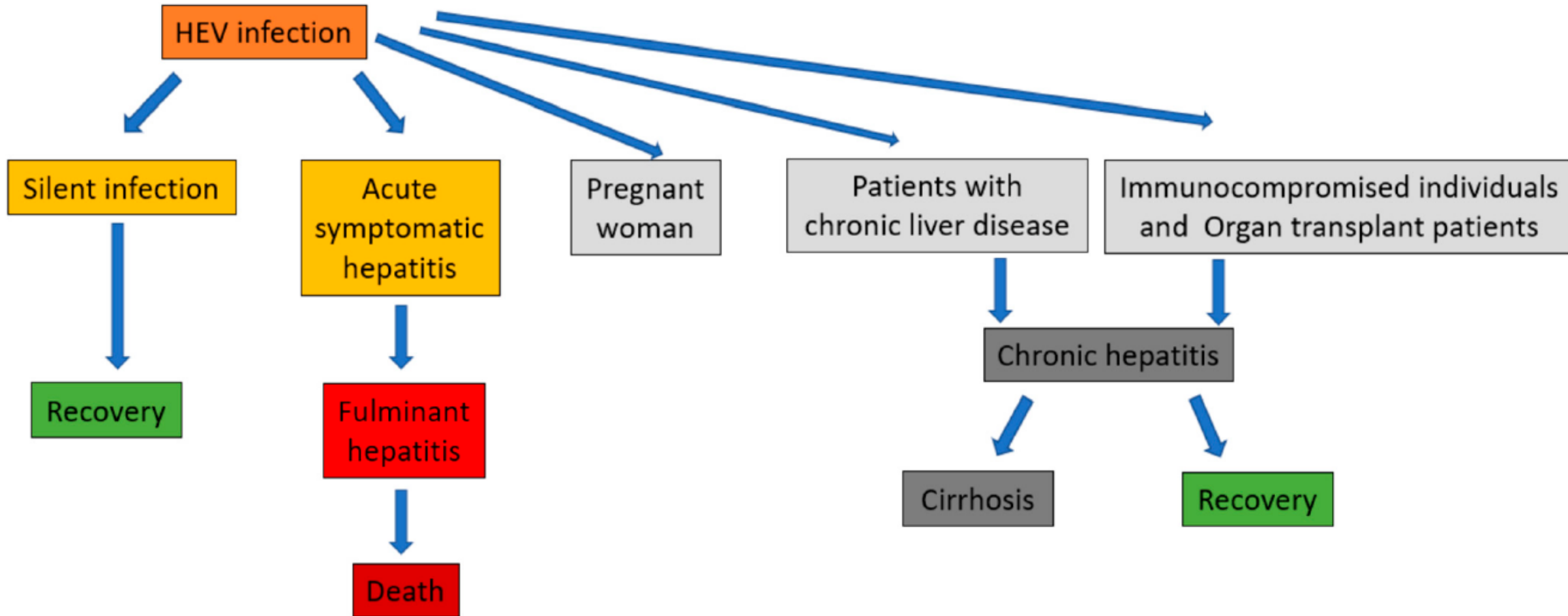


## Different disease scenarios seen with HEV





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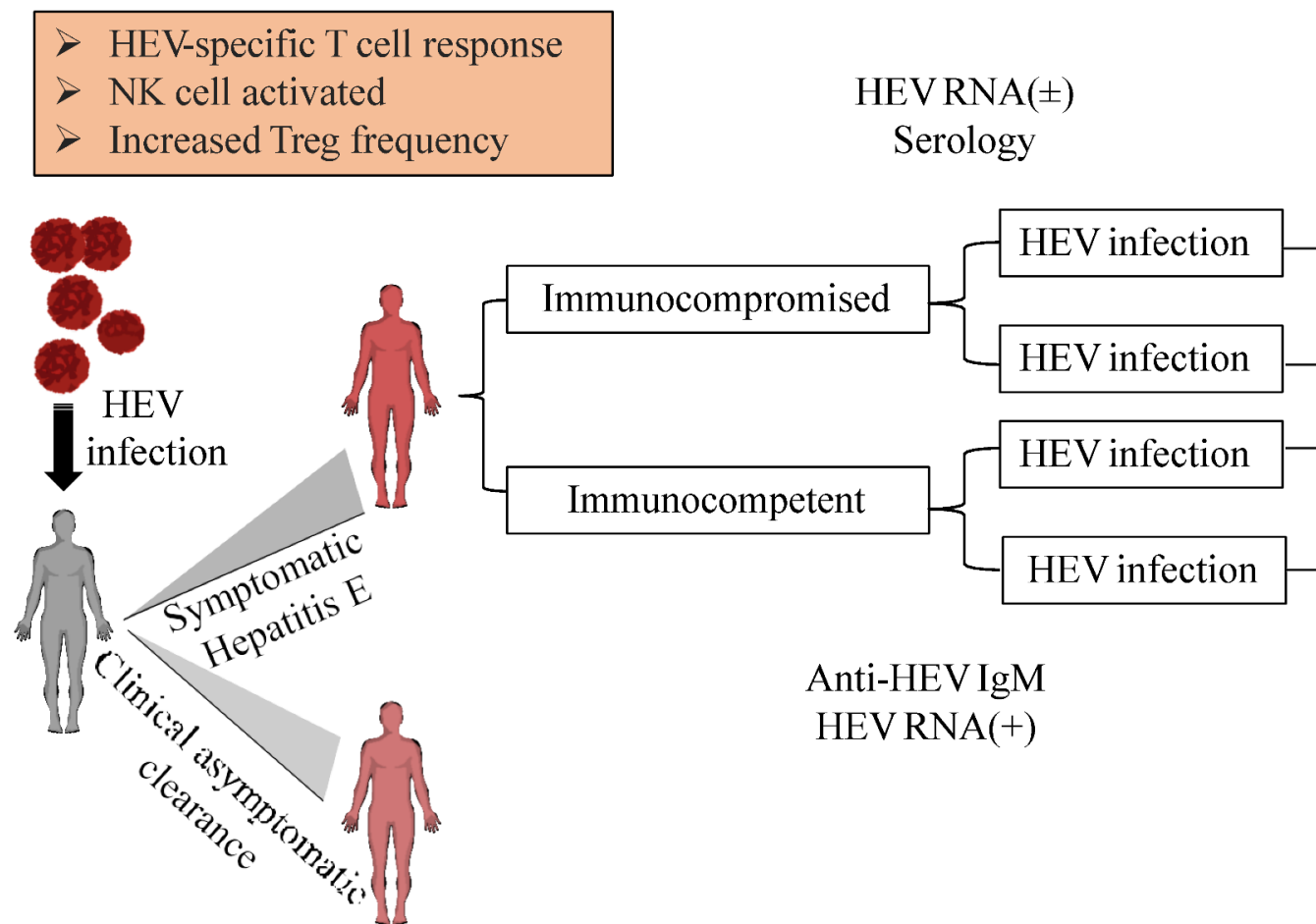
## Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation

Jurjen Versluis<sup>1</sup>, Suzan D. Pas<sup>2</sup>, Hendrik J. Agteresch<sup>1,3</sup>, Robert A. de Man<sup>4</sup>, Jolanda Maaskant<sup>2</sup>, Marguerite E. I. Schipper<sup>5</sup>, Albert D. M. E. Osterhaus<sup>2</sup>, Jan J. Cornelissen<sup>1</sup>, and Annemiek A. van der Eijk<sup>2</sup>

- High probability of developing chronic hepatitis
- HEV to be included in differential diagnosis of ALT elevation in HSCT

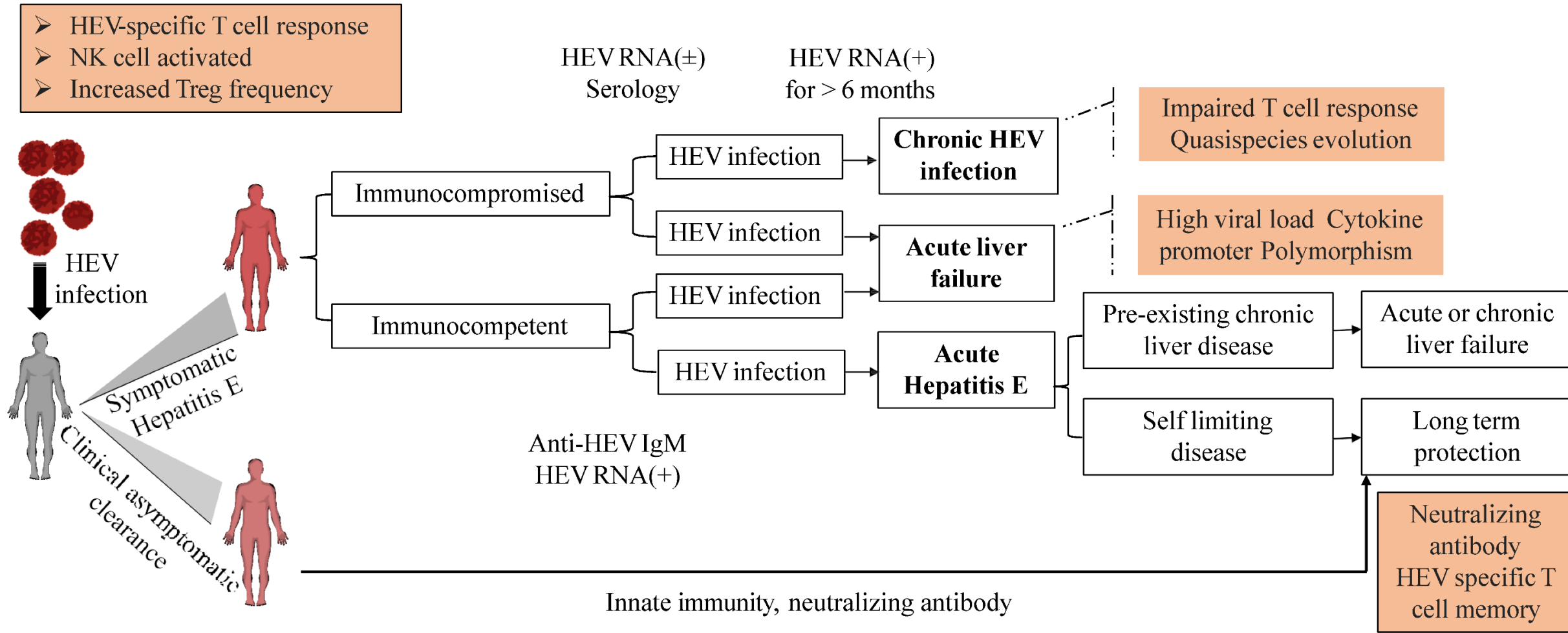
transplantation (alloHSCT). Therefore, we set out to study the incidence and sequelae of HEV as a cause of hepatitis in a recent cohort of 328 alloHSCT recipients. HEV RNA was tested in episodes of liver enzyme abnormalities. In addition, HEV RNA and HEV serology were assessed pre- and post-alloHSCT. We found 8 cases (2.4%) of HEV infection, of which 5 had developed chronic HEV infection. Seroprevalence pre-alloHSCT was 13%. Four patients died with HEV viremia, with signs of ongoing hepatitis, having a median time of infection of 4.1 months. The 4 surviving patients cleared HEV after a median period of 6.3 months. One patient was diagnosed with HEV reactivation after a preceding infection prior to alloHSCT. Although the incidence of developing acute HEV

# Patterns of hepatitis E virus infection manifestation



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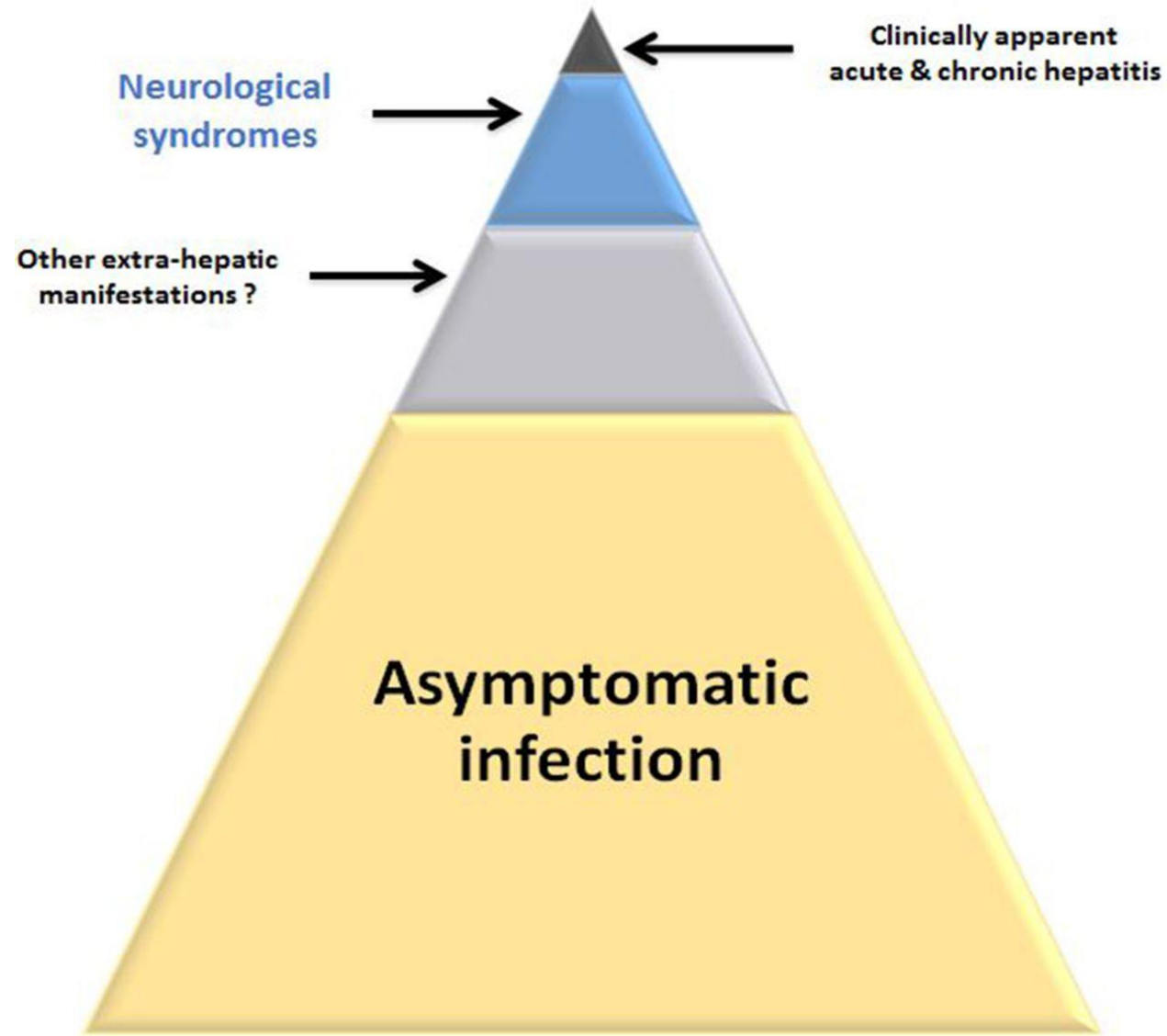
# Patterns of hepatitis E virus infection manifestation



# Outline

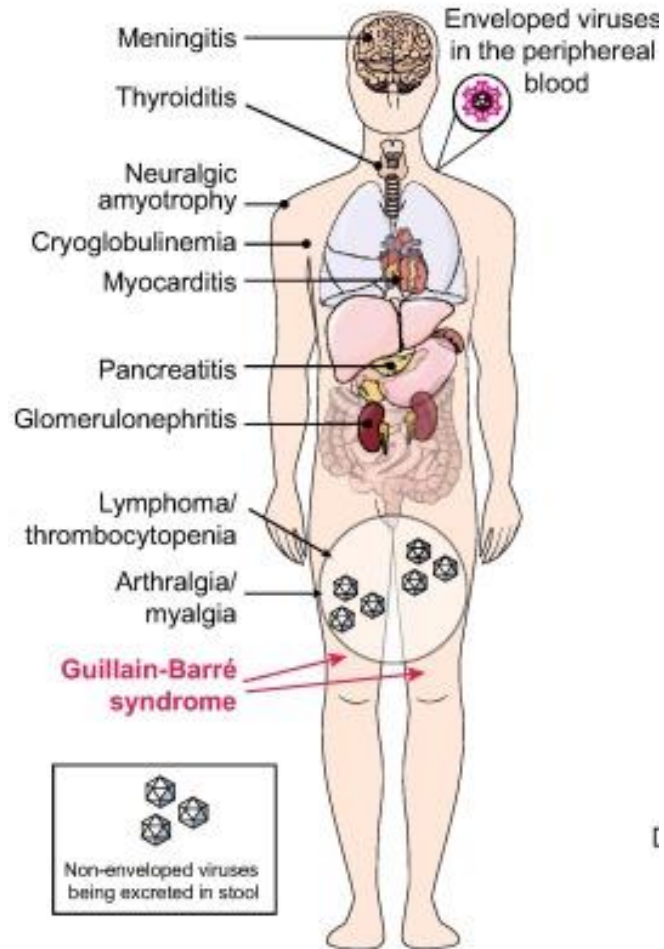
- HEV epidemiology: HEV is an emerging pathogen whose burden is largely underestimated due to underdiagnosis
- The biology of the virus (i.e. its quasi-enveloped nature) has been only recently partly elucidated
- Genotype-dependent differences in epidemiological and clinical patterns
- Extraepatic manifestations represent a major clinical and diagnostic challenge
- Pathogenetic studies hampered by scarce/inadequate study models
- Data from study models and from extreme situations in infected persons (pregnancy, transplant) indicate pathogenetic mechanisms triggered by both direct (virus replication-driven) and indirect (mostly innate immunity-driven) factors
- HEV has evolved multiple (in some cases genotype-dependent) mechanisms of evasion from innate immunity





**A**

**Reported extrahepatic organ  
manifestations in the context of  
hepatitis E virus infection**

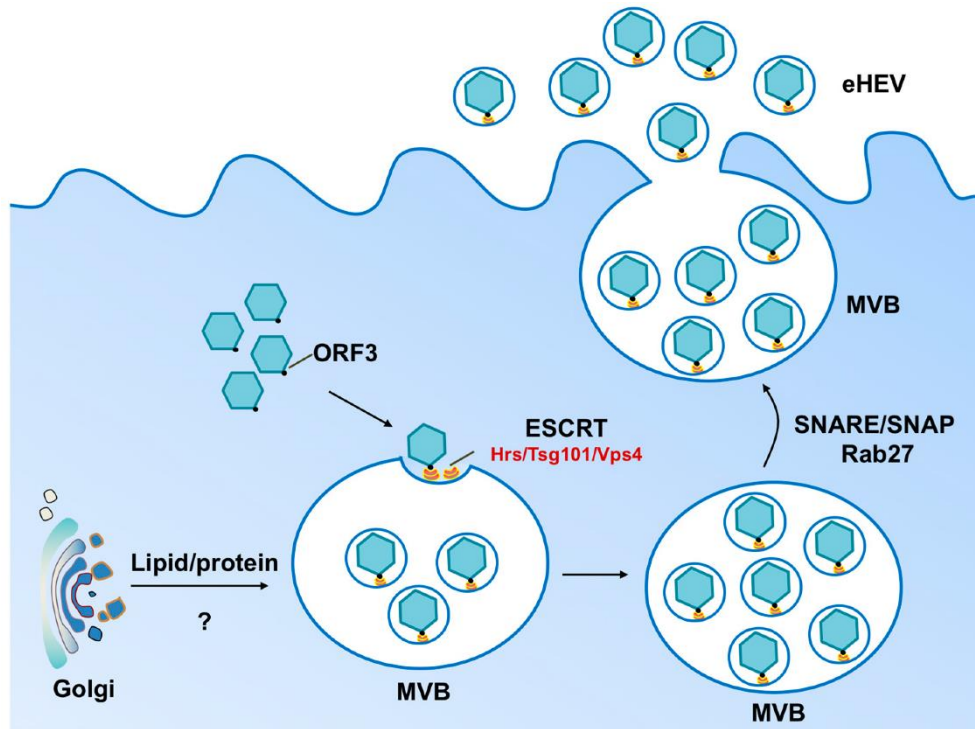


Several diseases have been observed in the context of hepatitis E, including

- Guillain-Barré syndrome
- neuralgic amyotrophy
- glomerulonephritis
- cryoglobulinemia
- pancreatitis
- lymphoma
- thrombopenia
- meningitis
- thyroiditis
- Myocarditis

To date, the definite pathophysiological links between HEV and extrahepatic manifestations are not yet established

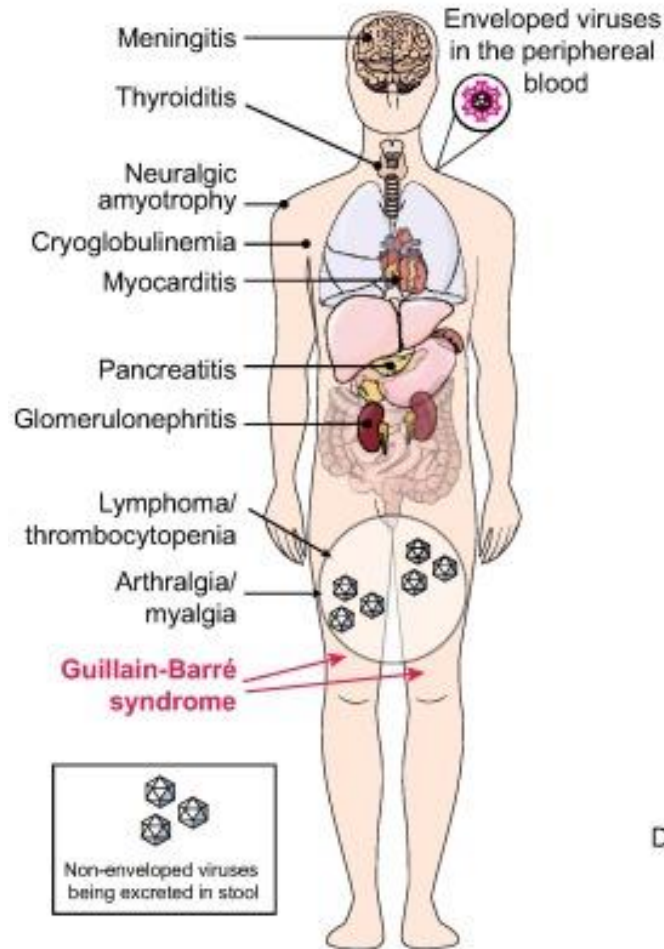
Causal relationship between HEV and neurological diseases has been proposed on the basis of significantly higher seroprevalence of HEV-associated neuralgic amyotrophy (NA) and Guillain-Barré syndrome (GBS) compared to non-HEV-associated cases. Moreover, several case studies have documented the presence of HEV RNA in the cerebrospinal fluid (CSF).



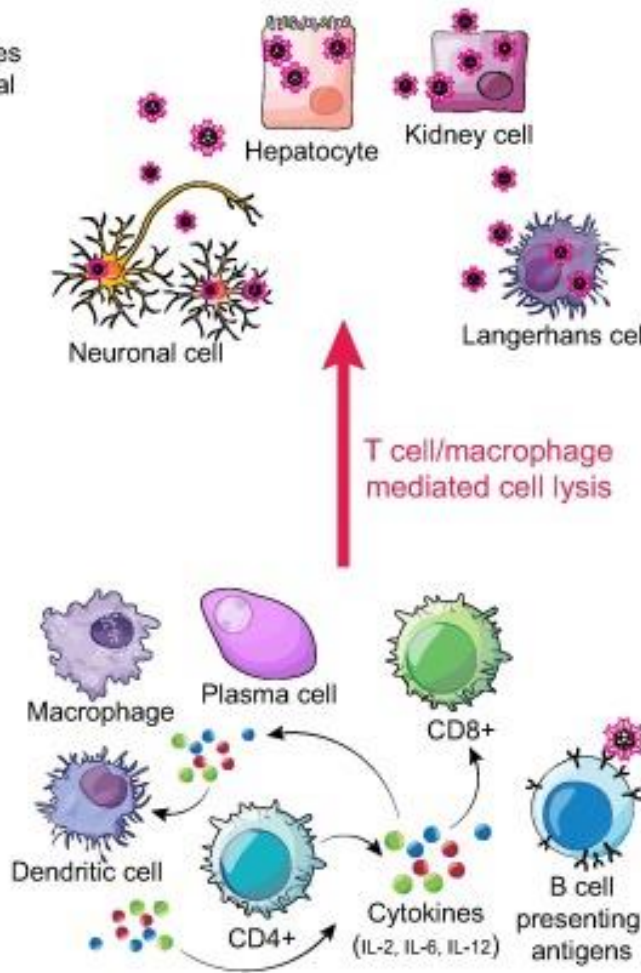
Because of its exosome-like quasi-envelope in the blood, eHEV could be taken up by different cell types through an endocytic process that does not depend on viral receptors

Similarities between the membrane-encased HEV virion and exosomes suggest the possibility that eHEV may penetrate immunologically privileged sites such as the central nervous system (CNS) as do exosomes

**A** Reported extrahepatic organ manifestations in the context of hepatitis E virus infection



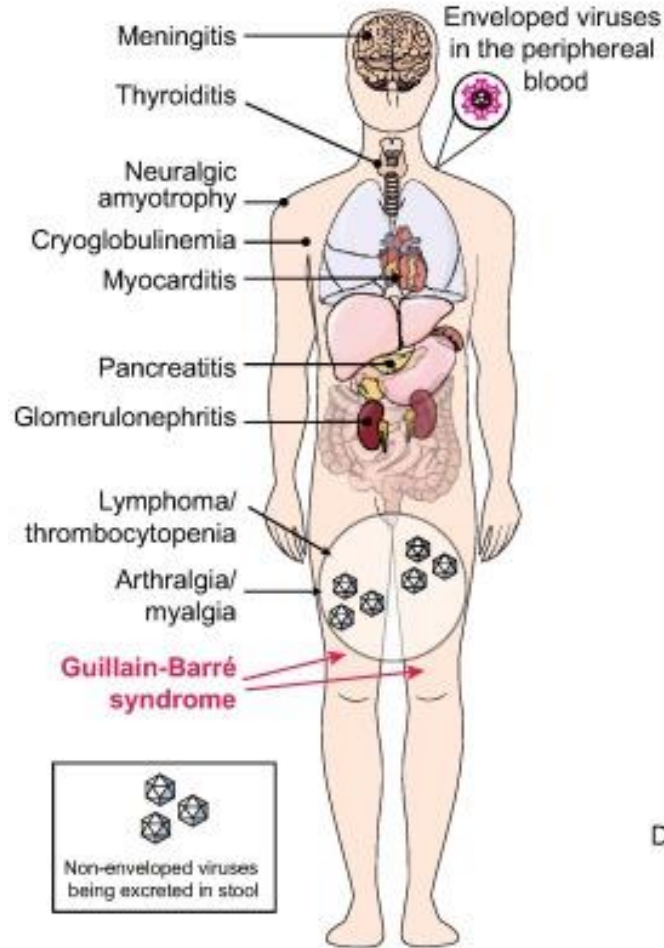
**B** Possible mechanisms of extrahepatic symptoms in the context of HEV replication



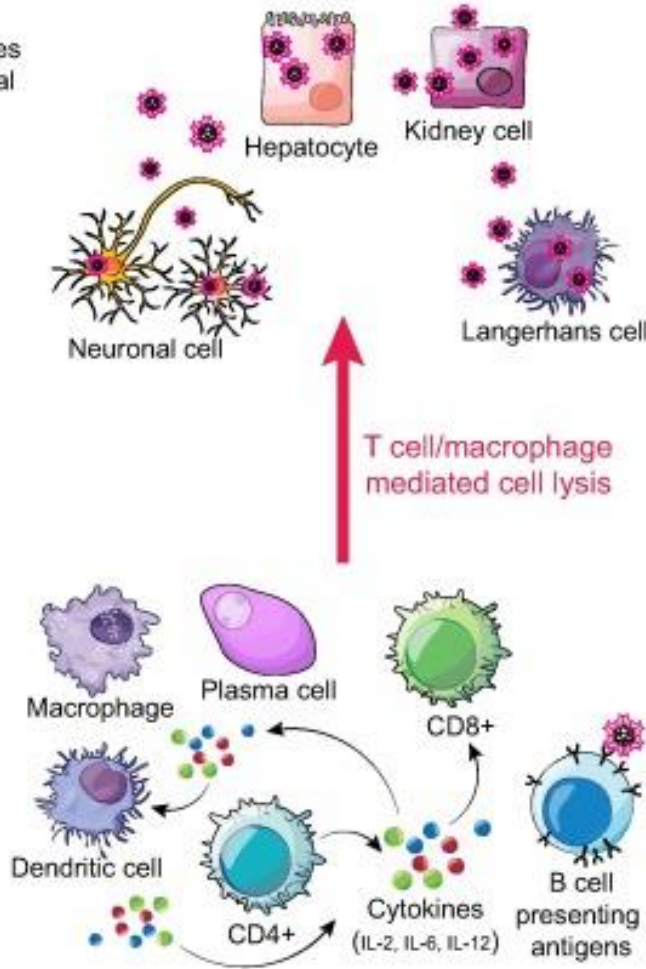
Either direct cytopathic tissue damage by **extrahepatic replication**, or **immunological processes** induced by an overwhelming host immune response, are possible origins of HEV-associated extrahepatic manifestations.



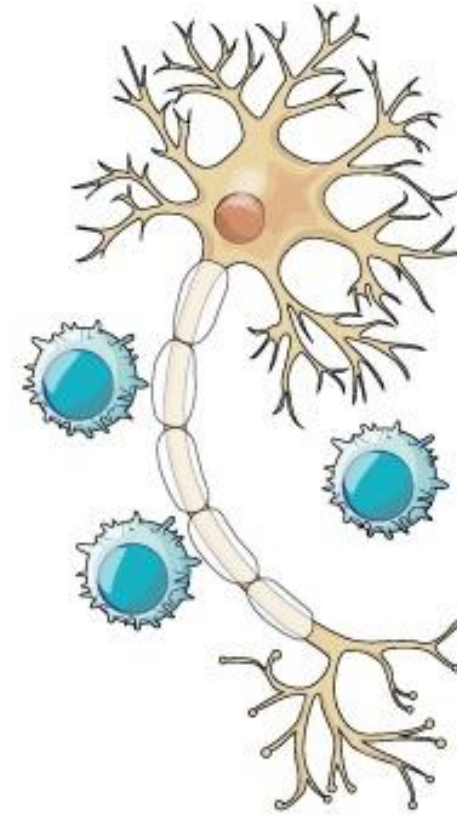
**A** Reported extrahepatic organ manifestations in the context of hepatitis E virus infection



**B** Possible mechanisms of extrahepatic symptoms in the context of HEV replication



**C** Possible mechanisms of neurological manifestations in the absence of HEV replication



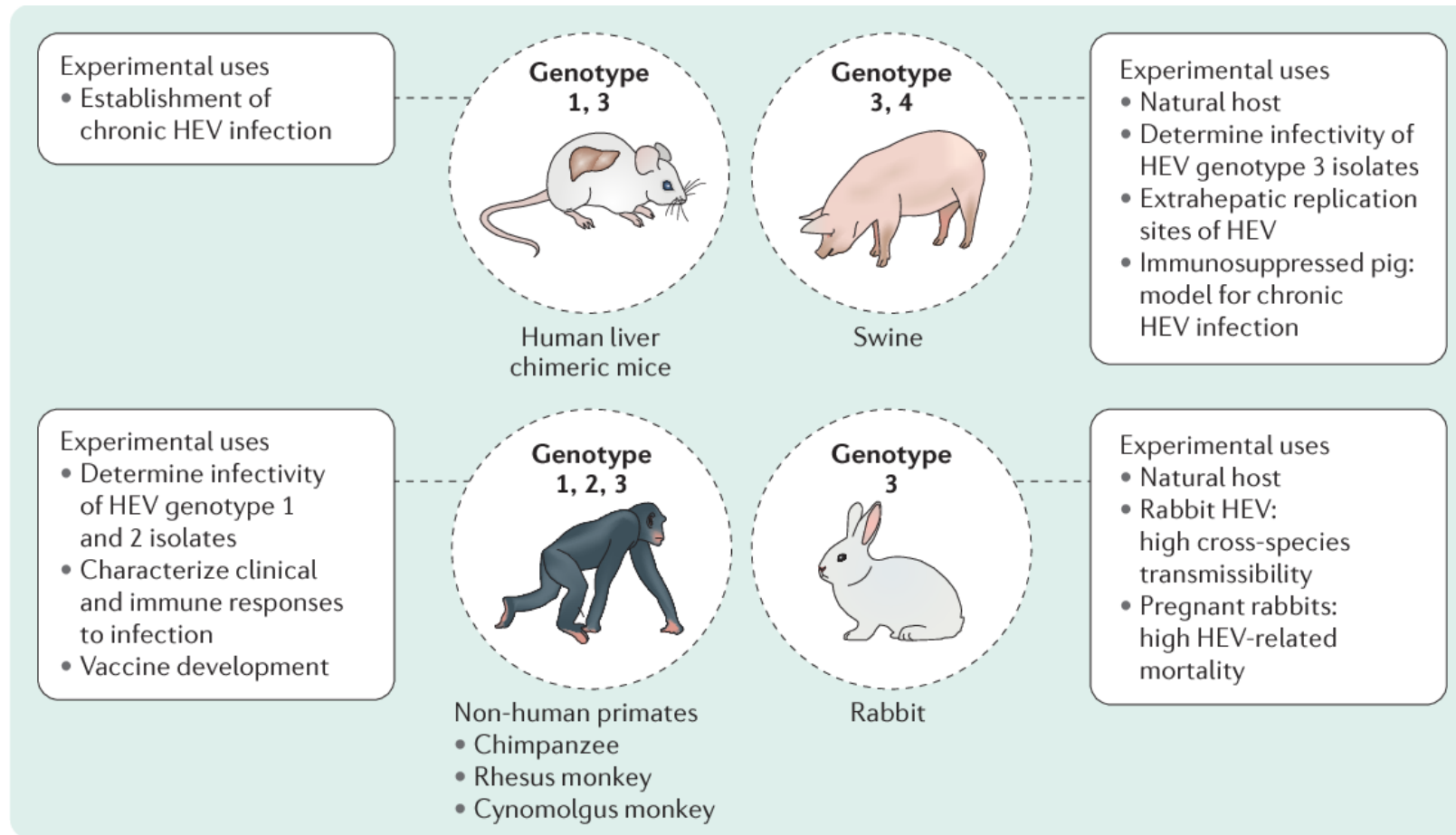


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Knowledge of the viral life cycle and pathogenesis is incomplete due to the limited availability of functional tools and study models

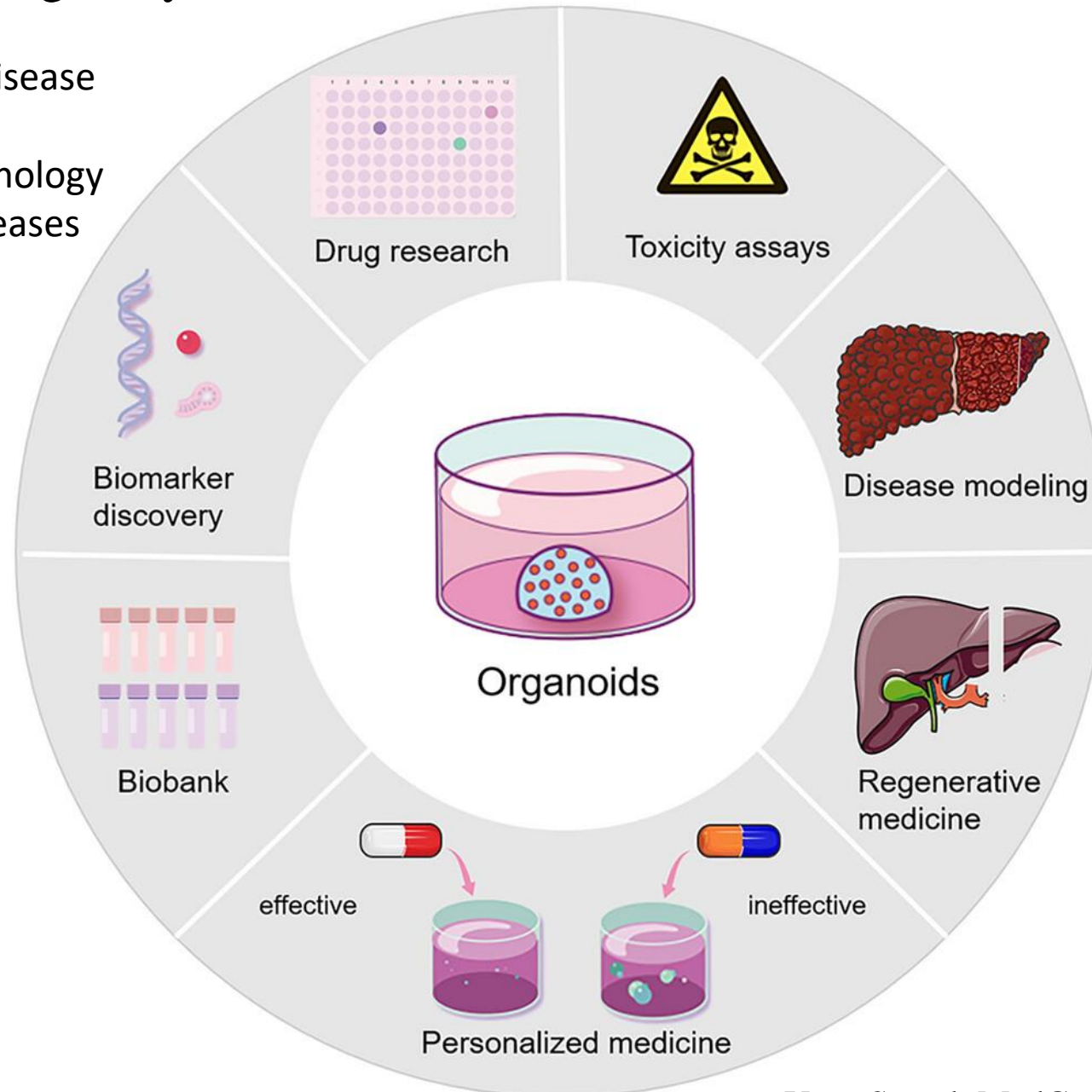
Current knowledge derives from reverse genetics systems, in vitro cell culture models, and representative studies in animal models including non-human primates



Experimental animal models to study HEV

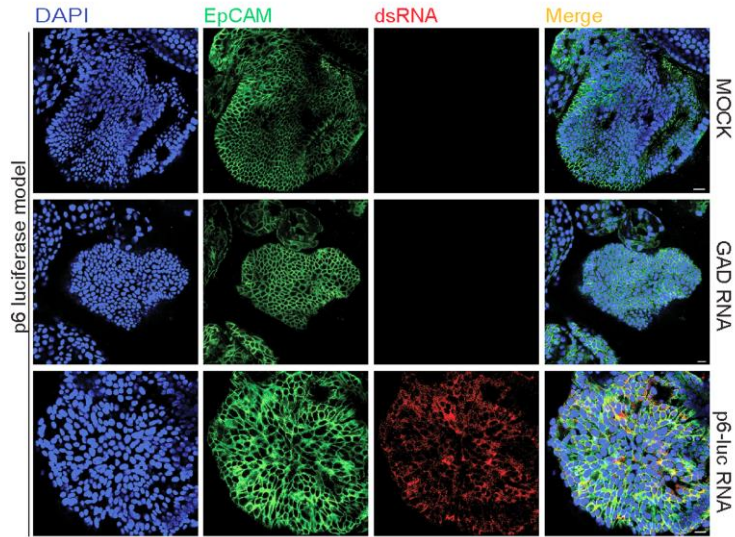
# Towards more physiologically relevant **2D** and **3D** cell culture models for studying HEV

Organoids can be used as disease models to understand the mechanisms and physiopathology of human hepatobiliary diseases

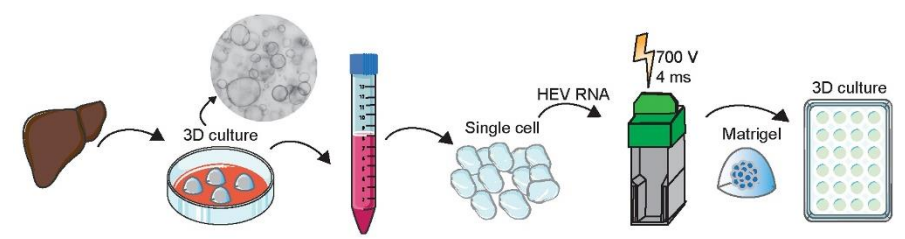
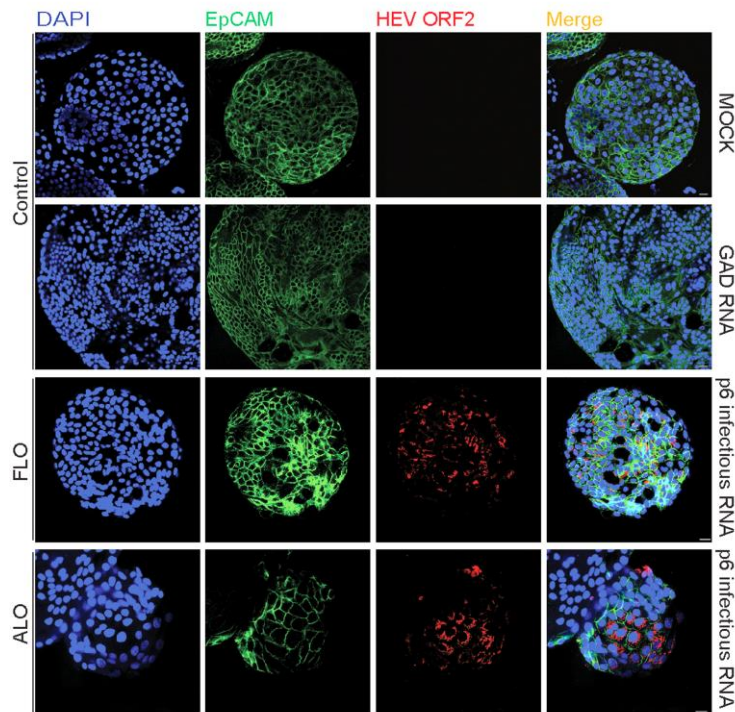


# Liver-derived organoids support the full life cycle of HEV infection

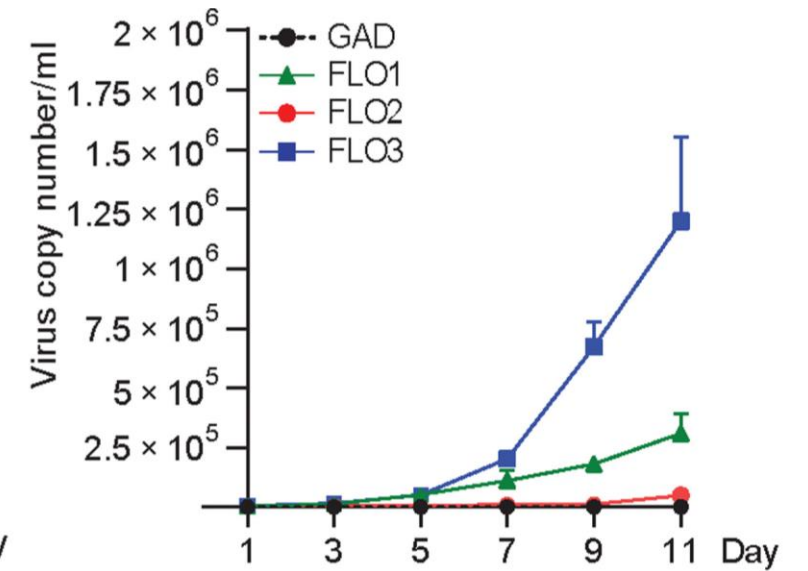
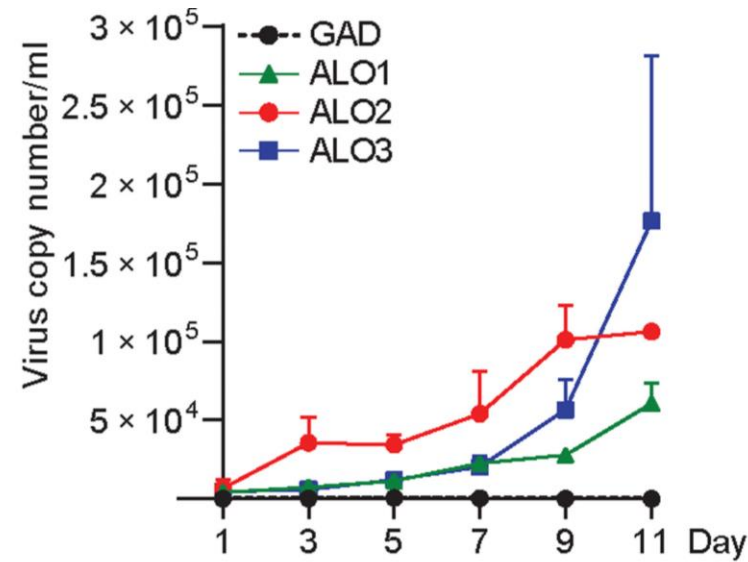
A



C



Released viral RNA increased gradually, finally reaching  $5 \times 10^4$ - $1 \times 10^6$  cp/ml in the six organoid lines. The released virus infected Huh7 hepatoma cells



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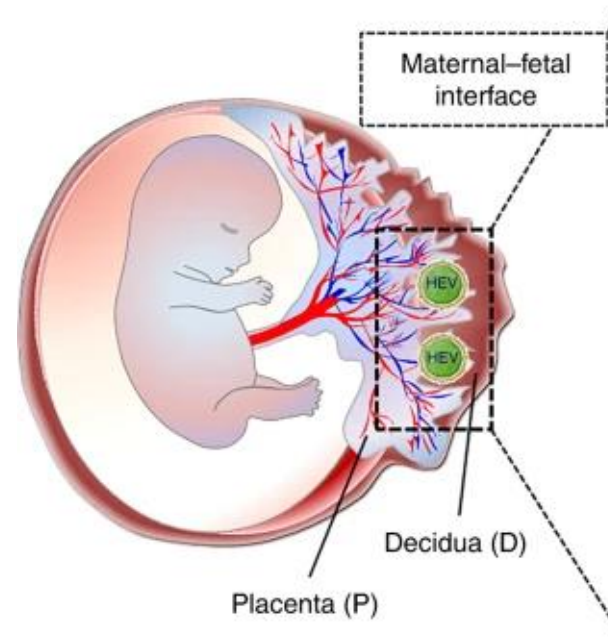
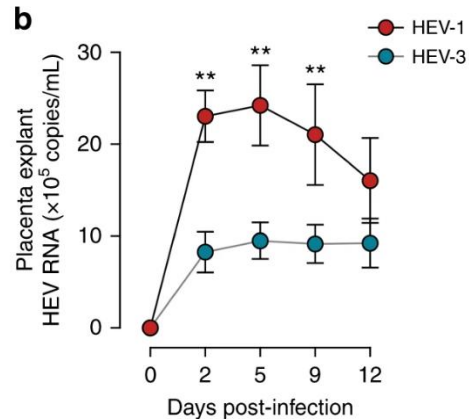
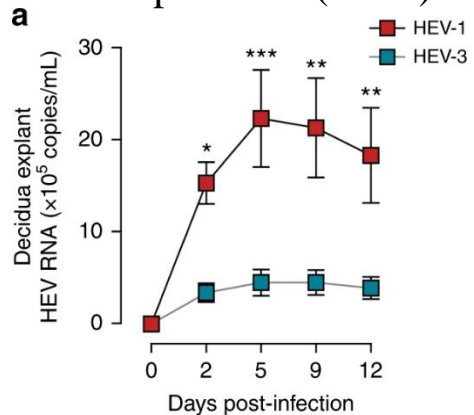
# HEV pathogenesis in pregnancy: gt3 vs gt1

- Infection with HEV gt1 during the third trimester can lead to maternal mortality in up to 15% to 25% of case
- Organ cultures from maternal decidua and fetal placenta explants were used to establish susceptibility to HEV-1 and HEV-3, to provide insights into HEV-1 pathogenesis during pregnancy

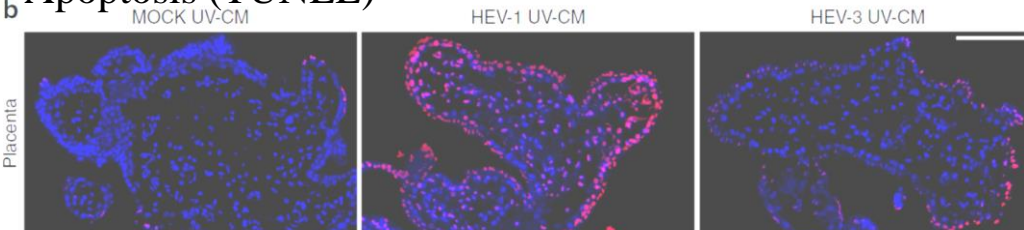
## Results:

- **HEV-1, but not HEV-3, replicates** to high level in both maternal and fetal tissues and causes tissue injury

### a Viral replication (RNA)



### b Apoptosis (TUNEL)



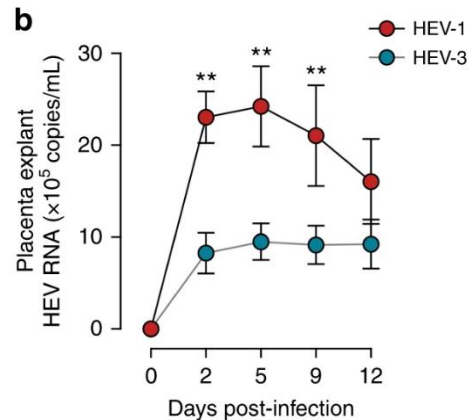
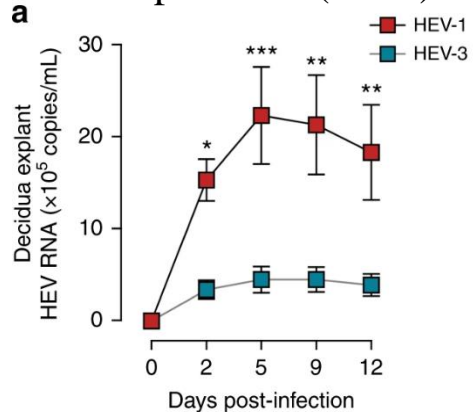
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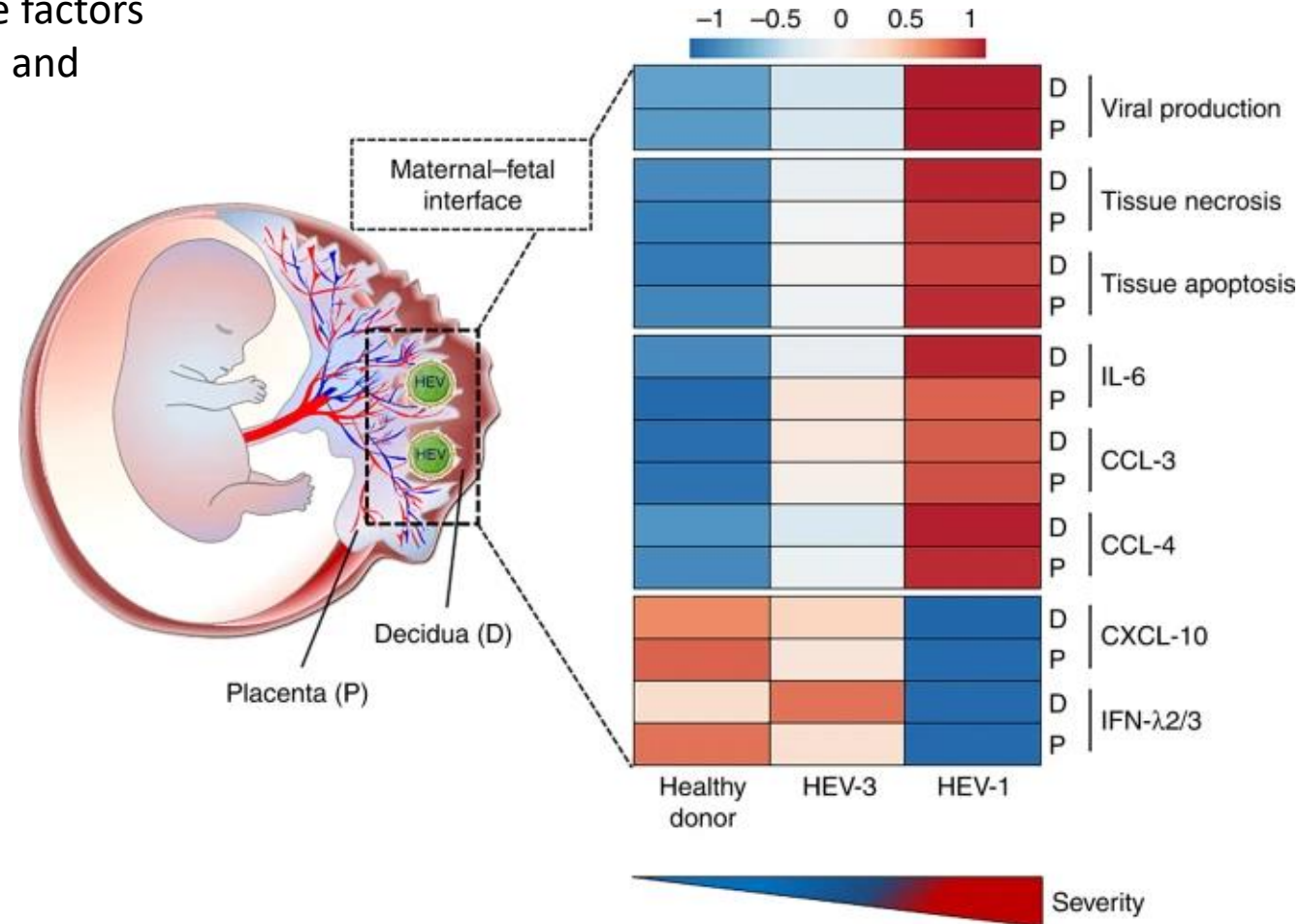
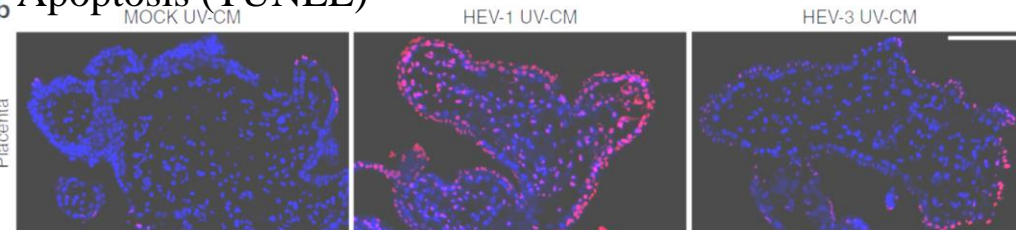
## Results:

- HEV-1, but not HEV-3, replicates to high level in both maternal and fetal tissues
- HEV-1 infection dysregulates the secretion of several soluble factors
- Altered cytokine microenvironment correlate with viral load and contribute to the tissue damage

### a Viral replication (RNA)



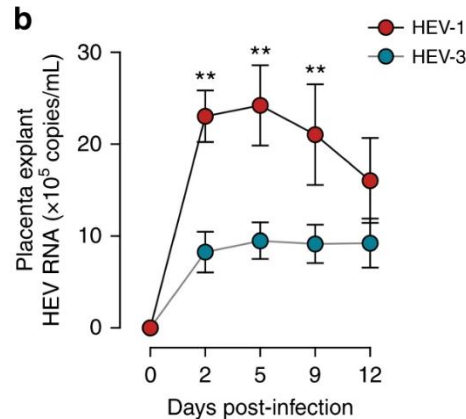
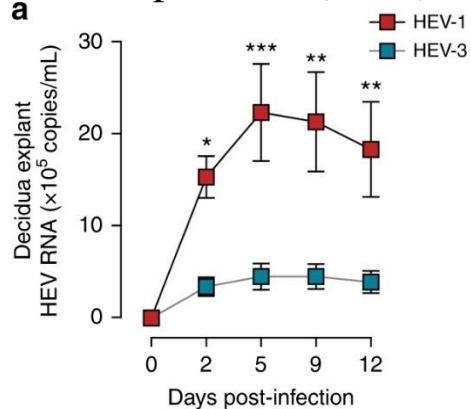
### b Apoptosis (TUNEL)



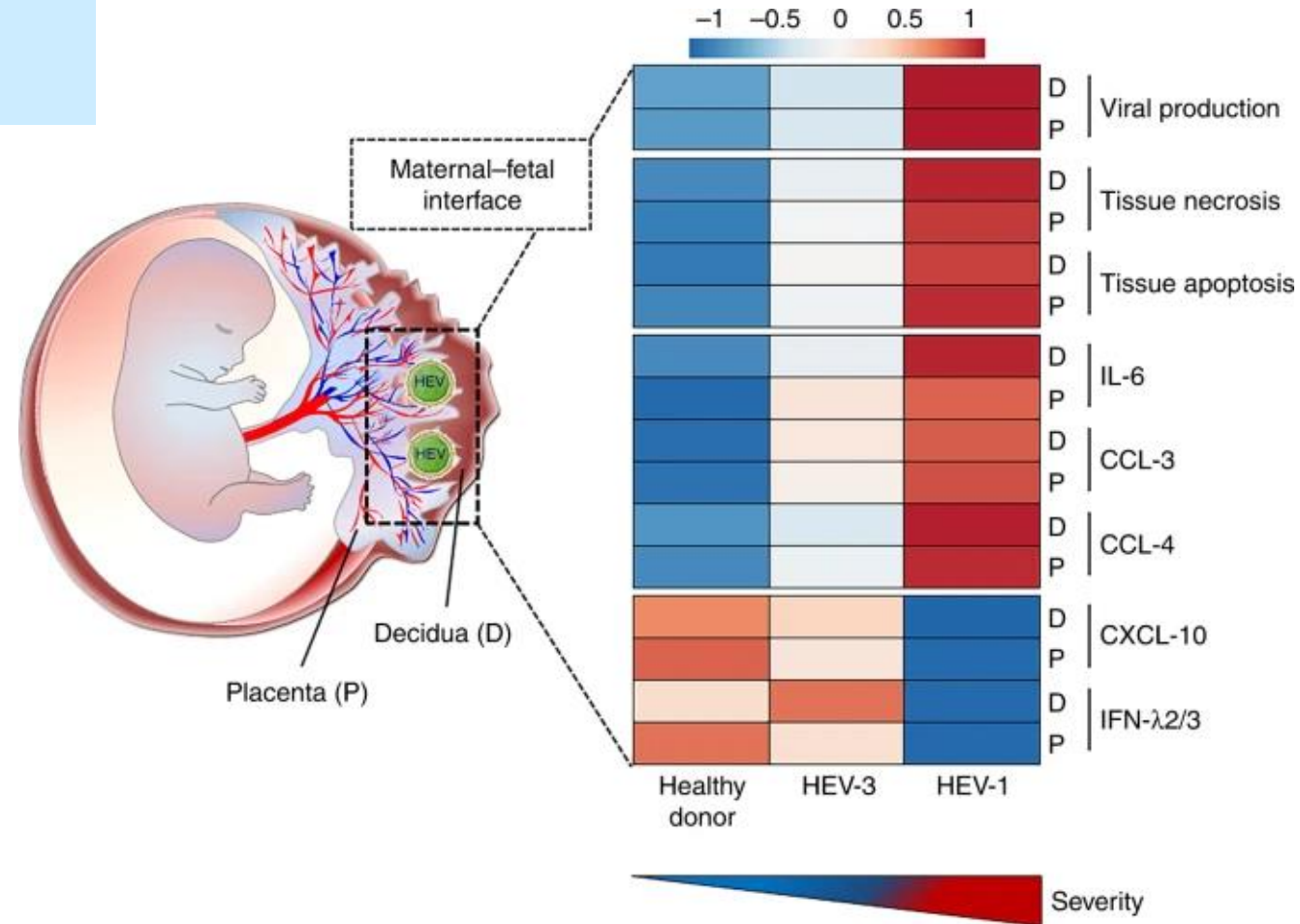
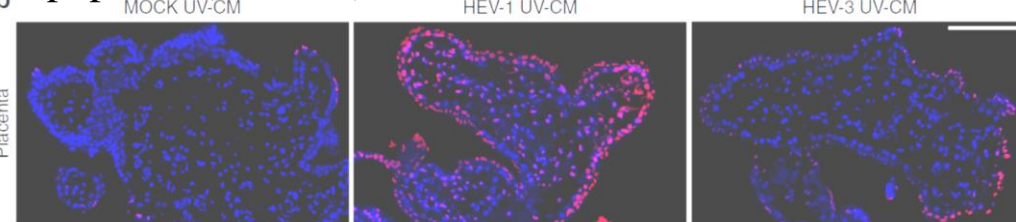
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- Organ cultures from maternal decidua and fetal placenta explants were used to establish susceptibility to HEV-1 and HEV-3, to provide insights into HEV-1 pathogenesis during pregnancy
- Collectively, HEV-1 pathogenesis during pregnancy is linked to
  - ✓ **high viral replication**
  - ✓ **alteration of the local secretome**
  - ✓ **induction of tissue injury**

## a Viral replication (RNA)



## b Apoptosis (TUNEL)



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# Hepatitis E virus - an emerging disease

## TRANSMISSION

Main routes



## SEROPREVALENCE



Established rates of anti-HEV IgG in humans



## PREVENTION

✓ HEV vaccine is approved only in China & Pakistan

The vaccine has 100% efficacy after the third dose and 96% after the second.

✓ Transfusion blood screening policy in 8 European countries

? Meat screening policies





THANK  
YOU!

