# Clinical case Mr L

No disclosure

# The patient

- Mr L., 41 years old
- Maried, 2 children
- Medical history :
  - Mononucleosis in 1992 with hepatitis
  - Depression in 1995 (no more treatment)
  - Liver Steatosis (LB in 1997 for elevated liver enzymes (gamma GT and ALT))
  - No hepatotoxic drugs

# Symptoms

- Asthenia
- Arthralgias, myalgias
- Fever (mild)
- Anorexia
- Intolerable Pruritus++++

- Biological tests (01/10/14) :
  - ALT 1837 U/ml
  - AST 216
  - PAL 469, GGT 1293
  - total Bil : 78 µmol/l
  - PT 105%

} Cholestasis

= Hepatitis?

- Clinical examination: icterus, dark urines

# What do you ask for?

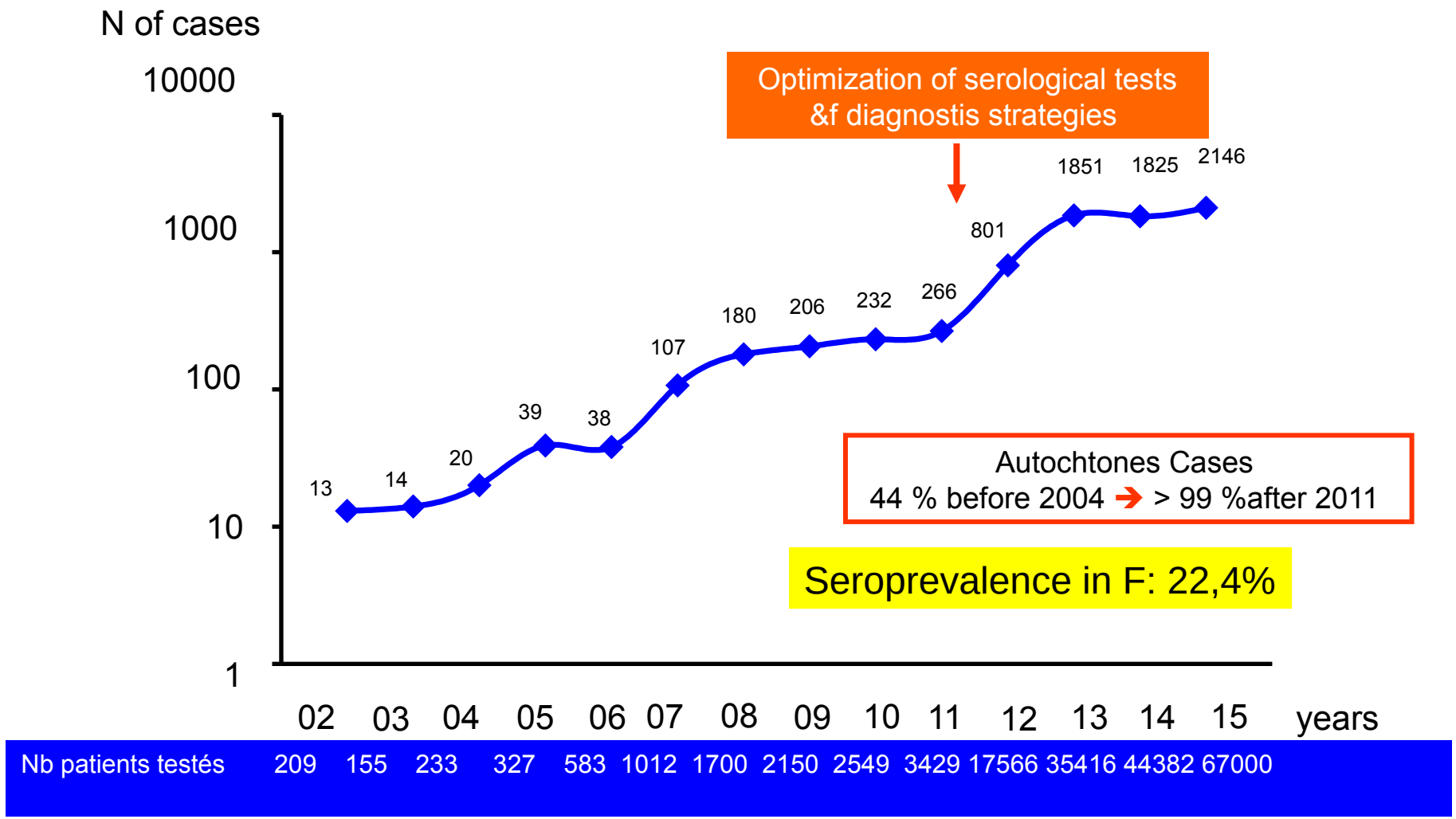
- Ultrasound : no biliary abnormalities

- Serological tests results

  - Hepatitis A : immunity ( tot Ab+, IgM -)
  - Hepatitis B : (HBs Ag-, anti HBs Ab-, anti HBc -)
  - Hepatitis C : HCV Ab -
  - EBV : IgG
  - CMV : IgG
  - Auto antibodies : negative

# Why screening for HEV?

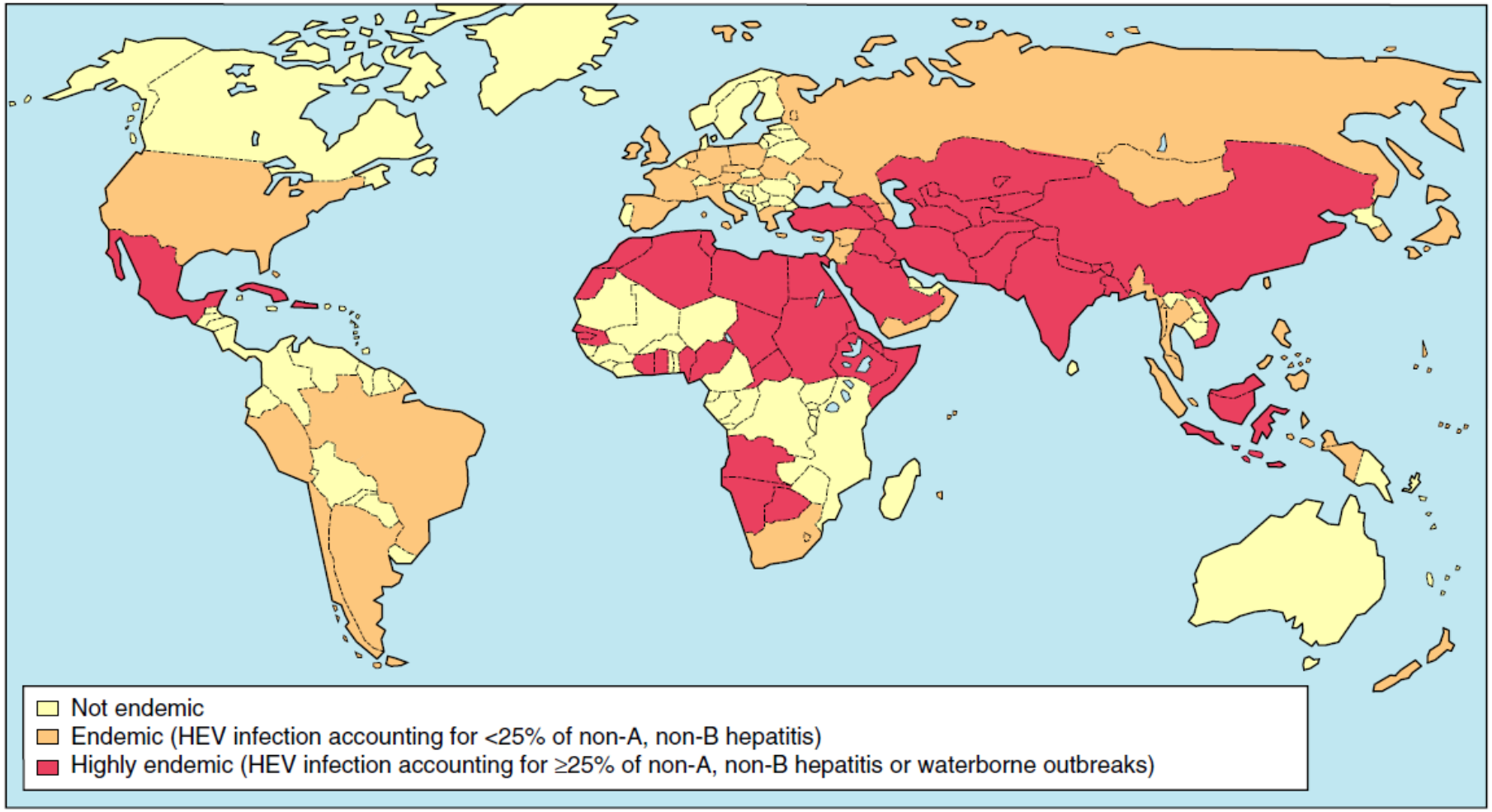
# Why screening for HEV?




# 1st cause of viral hepatitis in Europe

CNR VHE http://www.cnrvha-vhe.org

# HEV EPIDEMIOLOGY
## 1st cause of acute hepatitis?




Donnelly et al, APT 2017

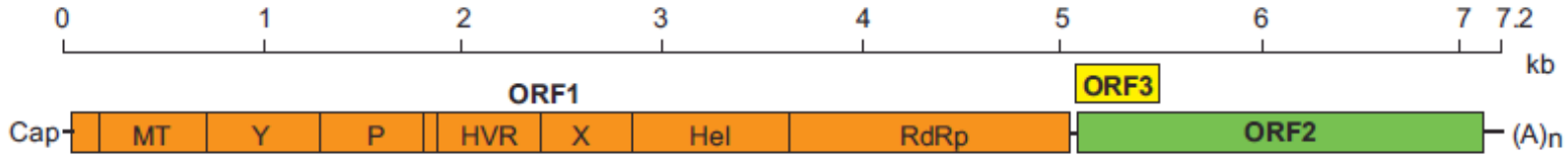
# Mr L.

| | 01/10/14 | 14/10/14 | 05/11/14 | 09/11/14 | 04/01/15 | 01/02/15 | 01/03/15 |
|---|---|---|---|---|---|---|---|
| IgG/M anti-HEV | - | + | + | + | + | + | + |
| PCR HEV serum | - | + | + | + | + | + Geno 3 | - |
| PCR HEV stools | | | | - | - | - | - |
| AST (U/l)<br>ALT (U/l) | 1216<br>1837 | 404<br>1135 | 45<br>67 | 46<br>63 | 20<br>32 | | |
| Bili tot. (µmol/l) | 78 | 139 | 361 | 492 | 44 | | |
| PAL (U/l) | 469 | 282 | 245 | 248 | | | |
| GGT (U/l) | | 364 | 83 | 72 | 100 | | |
| PT (%) | 105 | 116 | 91 | 84 | 95 | | |

- Follow up….
  - One month later : stable and improvement of liver tests
  - ALT : 962U/l, AST : 238
    - PAL : 331U:l, GGT : 614U/l
    - total Bili : 112 µmol/l,
    - **But still icterus and pruritus +++**
  - 3 months later : recovery

# HEV Structure

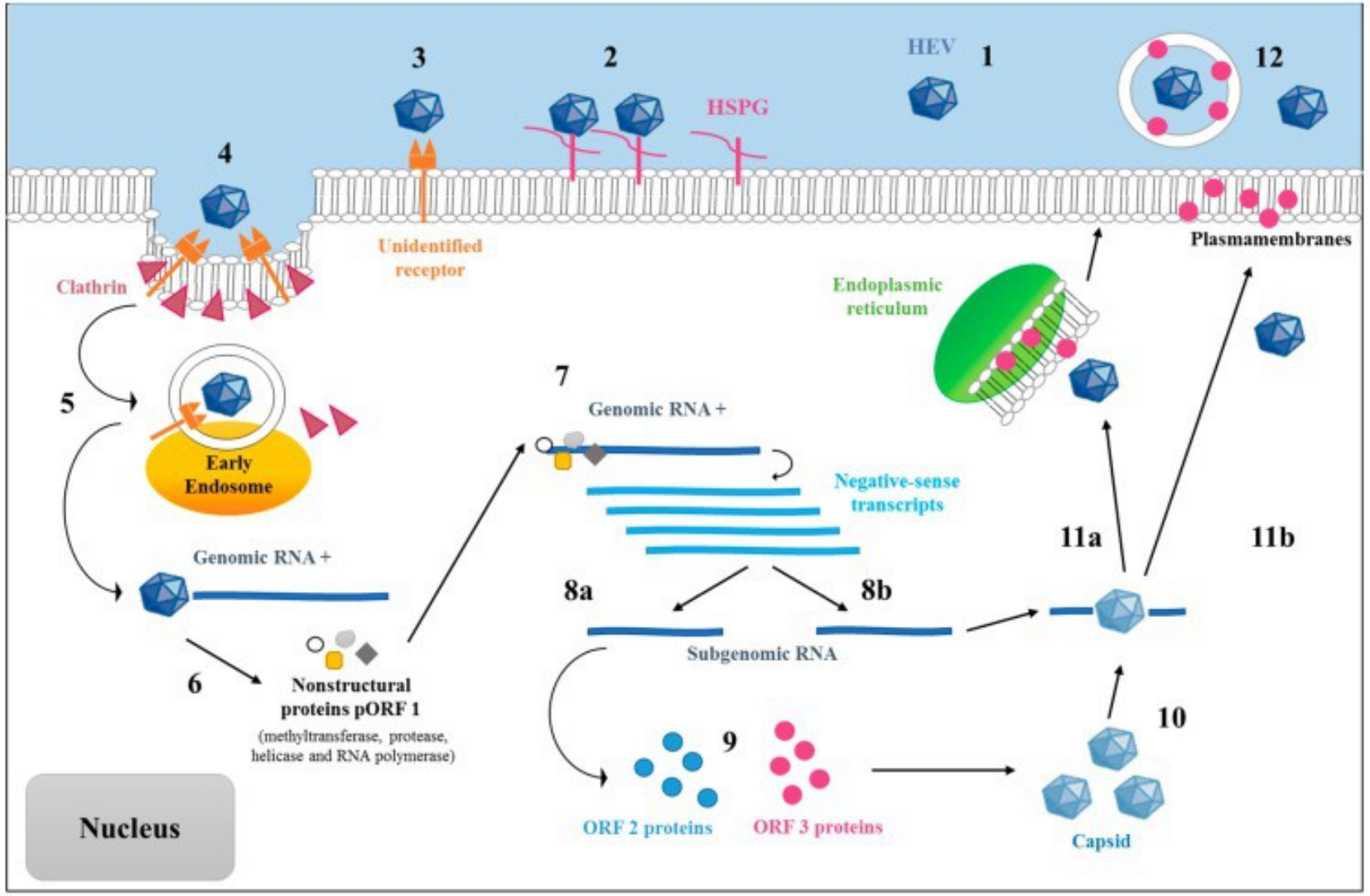
- 30 to 34 nm RNA non enveloped virus
- Virus ORF1 : non structural
- ORF2 : capsid
- ORF3 : protein associated to viral particules




***Okamoto, J Gastroenterol, J Gen Virol 2012***

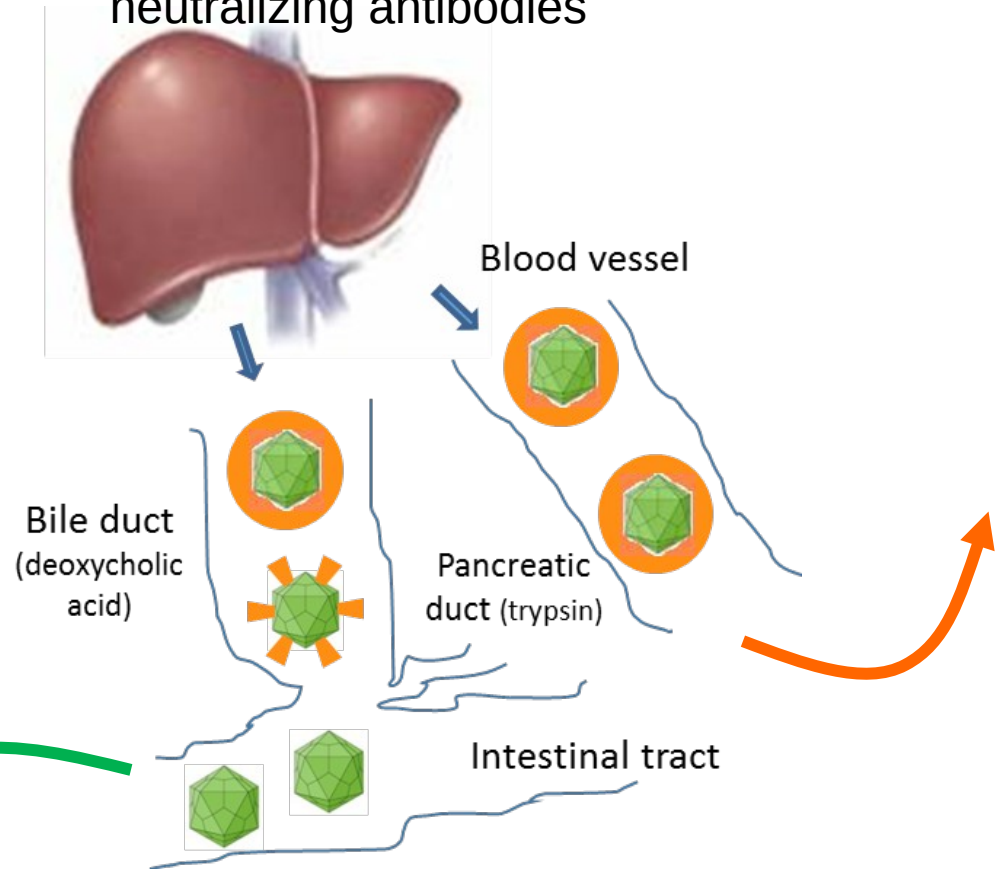
*Chapuy-Regaud, Biochimie, 2017*

- HEV replication cycle



# Propagation in vivo

Cell entry, cellular tropism and infectivity, escape to neutralizing antibodies




**Mature virions are released attached to ORF 3 protein and lipids (quasi-enveloped form in bloodstream)**
Propagation (out of sight of immune response) and infection of other organs

viral particules are **free in bile** and released outside

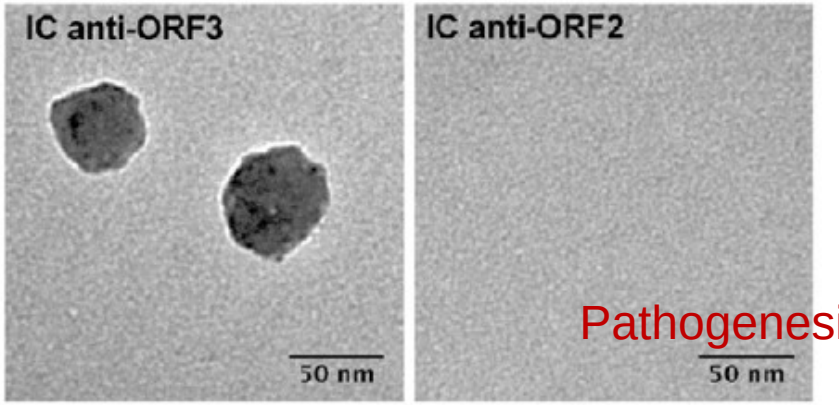
***Okamoto, J Gastroenterol, J Gen Virol 2012***

*Chapuy-Regaud, Biochimie, 2017*

# Viral Particule

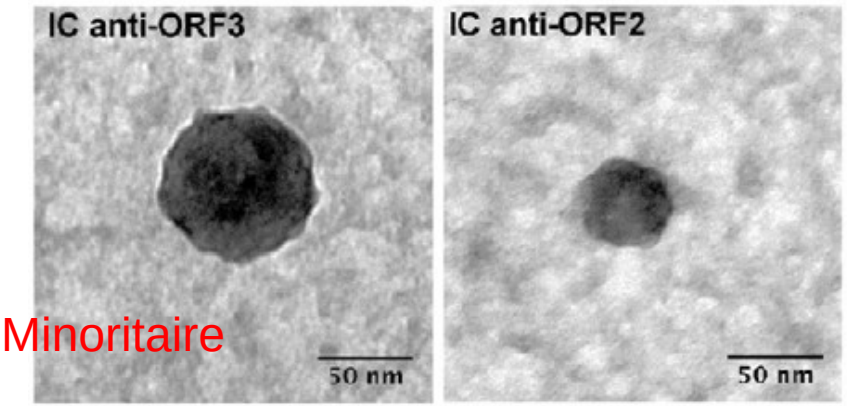
Surnageant Culture

Virus des cultures

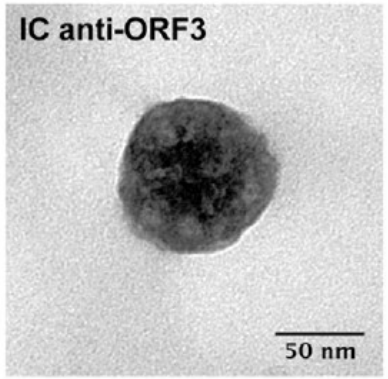



Pathogenesis

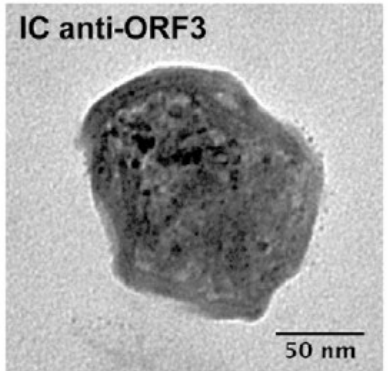
Virus des cultures
Traité par détergent



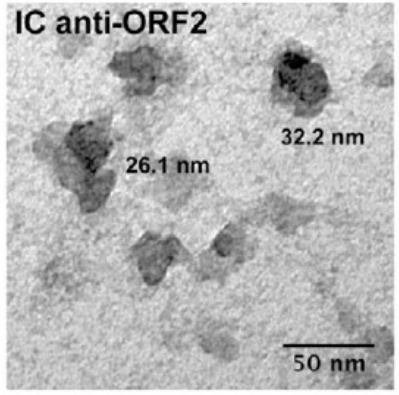

Minoritaire

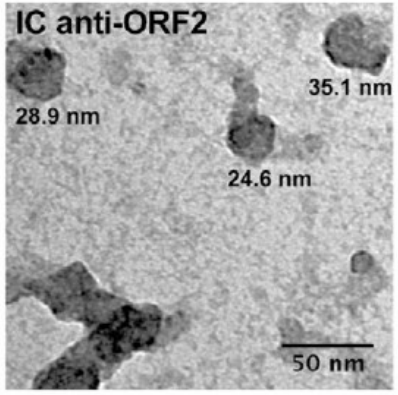



Serum




stools



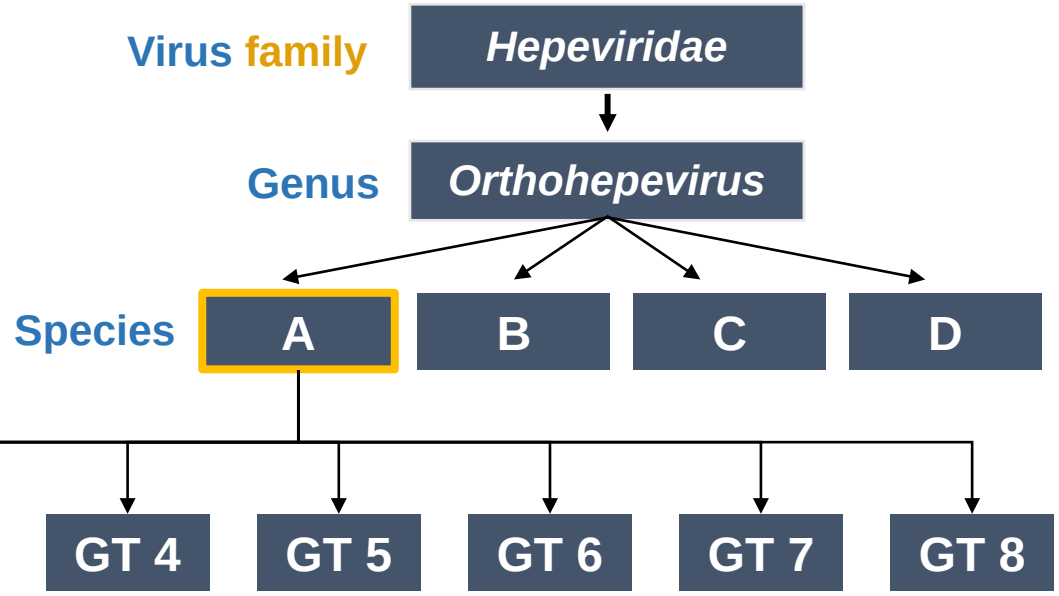

*Montpellier, Gastroentrology 2017*

# Variability of HEV

*Hepeviridae* viruses infect mammals, birds and fish

Strains infecting humans belong to the *Orthohepevirus* genus, species A

Species A comprises **8 genotypes**




- Only infect humans
- **Faecal–oral spread** via contaminated water
- Large **outbreaks**
- **Developping countries**
- Brief, **self-limiting**
- Never chronic
- High mortality in **pregnancy** (25%)

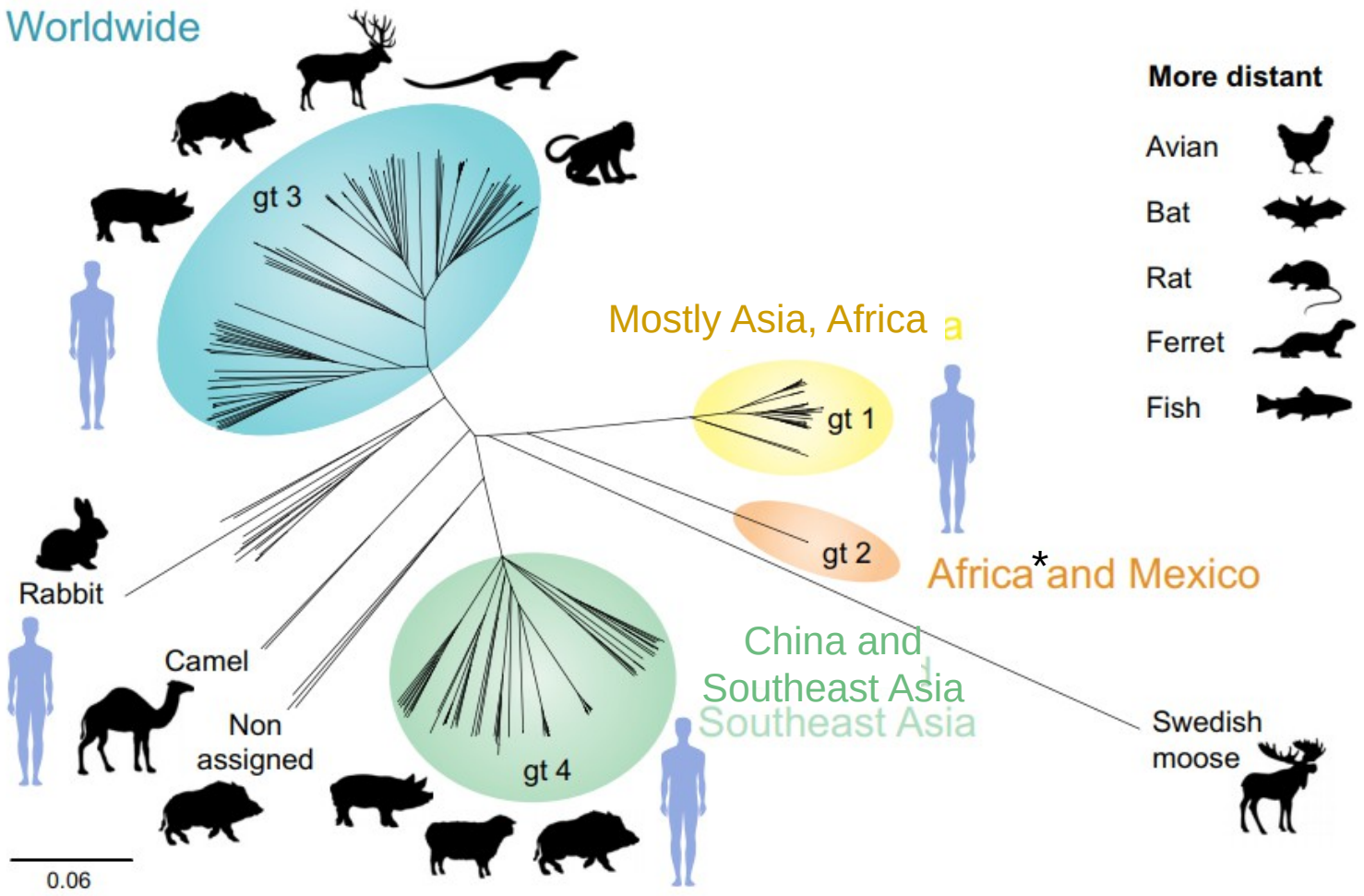
- **Endemic** in animal species; eg, pigs and wild boar
- **Zoonotic** infections in humans
- **High-income countries**
- China: GT 4 most common
- S. America: GT 3 only

- Have only been reported in wild boar

- GT 7 identified in patient regularly consuming camel meat and milk
- Have since been identified in camels

EASL CPG HEV. J Hepatol 2018;doi: 10.1016/j.jhep.2018.03.005 [Epub ahead of print]

# Phylogenetic relationship of hepeviruses




* Namibie and Nigeria

Debing Y, et al. J Hepatol 2016;65:200–12

# Pathogenesis

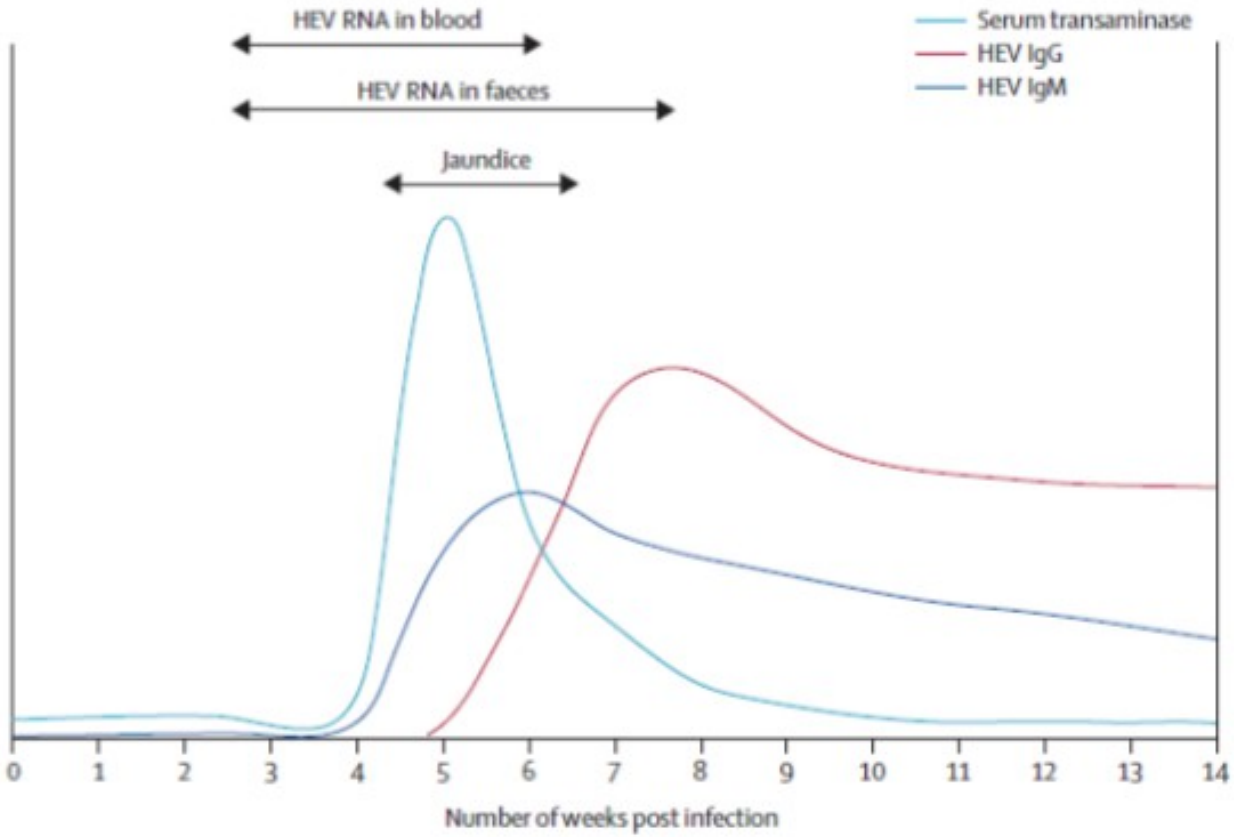
✓ Most of infections are asymptomatic

Rein, *Hepatology* 2012 ; Said, *Emerg Infect Dis* 2009;
Guillois, *Clin Infect Dis*, 2015 ➔ 70%

| Developing countries | High income countries |
|---|---|
| Genotype 1-2 | Genotype 3-4 |
| Young adults 15-35 ans | Adults > 55 years |
| men | men |
| Fulminant forms in pregnant women or in patients with chronic liver diseases<br>**No chronicity** | Fulminant forms in patients with chronic liver diseases<br>**Chronic forms** |
| **Cholestatic forms? Not described?** | **Cholestatic forms? Not described?** |
| Khuroo, *J Viral Hep 2003* | Dalton, *Lancet 2007* ; Peron, *J Viral Hep 2007* |

# Diagnosis - Sequence of biological and virological events after acute infection

- Serological tests
- RT-PCR Genotype

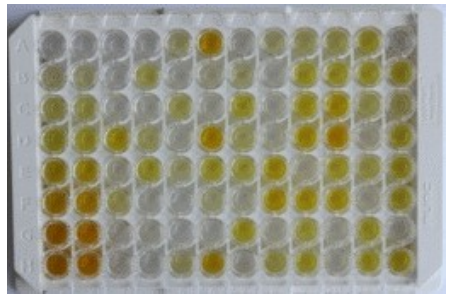


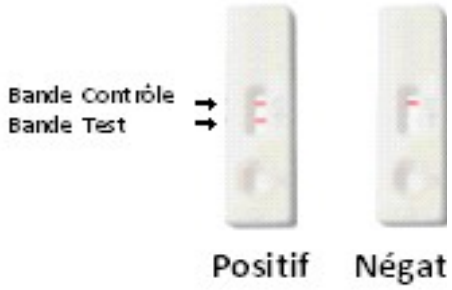

Représentation schématique de l'évolution des marqueurs biologiques lors d'une infection virale E.

# Serologic Tests

✓ **anti-HEV IgM**

➔ **High Sensitivity and specificity**






**Sensitivity :**

Immunocompetents 98%

Immunosuppressed 80-85%

**Rapid test**

**Sensitivity :**

Immunocompetents 90%

Immunosuppressed : 70%

✓ **anti-HEV IgG**

➔ **Heterogenous Performances**

➔ **LOD 0,25-2,5 WHO UI/ml**

**Bendall, *J Med Virol* 2010 ; Mansuy, *Emerg Infect Dis* 2011,** Pas, *J Clin Virol* 2013
**Abravanel, *J Clin Virol* 2013; Norder *J Clin Microb* 2015**

# New tests

✓ **VIDAS ® IgG et IgM anti-HEV, Biomérieux**

➔ **Sensitivity IgG 0,56 U/ml**

| | | Infectious profile | | | |
|---|---|---|---|---|---|
| | | Viremic phase | Post-viremic phase | No recent infection | Total |
| **VIDAS®** | **Positive** | 83 | 42 | 2 | 127 |
| **Anti-HEV IgM** | **Négative** | 2 | 29 | 301 | 332 |
| **Total** | | 85 | 71 | 303 | 459 |
| **Performances** | | % | [IC95%] | | |
| **Positive concordance for viremic phase (acute phase)** | | **97.65 %** | [ 91.76 ; 99.71 ] % | | |
| **Positive concordance for post-viremic phase** | | ***59.15 %*** | [ 47.54 ; 69.83 ] % | | |
| **Negative concordance** | | **99.34 %** | [ 97.64 ; 99.92 ] % | | |

***Abravanel, ESCV 2017***

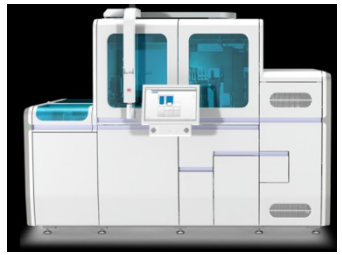
✓ **VIRCLIA ® / ALEGRIA ® IgG et IgM anti-HEV, Orgentec**

**Performance ???**


# Direct Tests

✓ **HEV RNA: PCR or TMA, LoD 10-60 UI/ml**

Abravanel, *J Clin Microbiol* 2013 ; Gallian, *Transfusion* 2017



Cobas 6800
Roche ®


Procleix Grifols ®,
Panther Hologic ®

Qualititative

Real Star HEV V2 Altona®
Quantitative

✓ **HEV Ag** specificity : 100 %
sensitivity: 91 %

80 % immunocompétent

94 % immunosuppressed

analytical sensitivity < PCR or TMA

Wen, *J Clin Microbiol* 2015 ; Trémeaux, *J Clin Virol* 2016 ; Behrendt, *J Infect Dis* 2016

# HEV markers according to infection status

| Infection status | Positive markers |
|---|---|
| **Current infection – acute** | • HEV RNA<br>• HEV RNA + anti-HEV IgM<br>• HEV RNA + anti-HEV IgG*<br>• HEV RNA + anti-HEV IgM + anti-HEV IgG<br>• Anti-HEV IgM + anti-HEV IgG (rising)<br>• HEV antigen |
| **Current infection – chronic** | • HEV RNA (± anti-HEV) ≥3 months<br>• HEV antigen |
| **Past infection** | • Anti-HEV IgG |

*Patients with re
positive

EASL CPG HEV. J Hepatol 2018;doi: 10.1016/j.jhep.2018.03.005 [Epub ahead of print]

# Molecular tests indications

- **Diagnosis of acute infection in seronegative patients**
  **immunocompetent patients** ***Mansuy JCM 04***
  **immunosuppressed patients** ***Kamar NEJM 08, Dalton NEJM 2010***

- **Diagnosis of persistent infection in immunosuppressed patients**
  - **HEV RNA persistance 3-6 months**

- **Follow up of treatment**
  **➔ blood and stools**

- Family of Mr L.

- His wife had asthenia and children had symptoms
  - His wife : 32 years old : asthénia ++ : AST 75 U/l, ALT 110 U/l)
  - chidren :
    - Kerrian, 5 years : asthenia, anorexia, fever ALT: N, → 12 months : fever ,
    - Maden
- What are you suspecting and why???
- Any suggestion??

# Mrs L.

| | 19/10/14 | 27/10/14 | 09/11/14 | 04/01/15 | 01/02/15 | 01/03/15 |
|---|---|---|---|---|---|---|
| IgG and IgM anti-HEV | - | - | - | + | + | + |
| PCR HEV serum | | | + | + | + weak | - |
| PCR HEV stools | | | - | + | - | - |
| AST (U/l) ALT (U/l) | 20<br>97 | 12<br>29 | 9<br>19 | 16<br>28 | | |
| Bili tot. (µmol/l) | 5 | 5 | | 7 | | |
| PAL (U/l) | 57 | 67 | 63 | | | |
| GGT (U/l) | 15 | 17 | 13 | 14 | | |
| TP (%) | 102 | 100 | 95 | 98 | | |

# L. Kerrian

| | 27/10/14 | 10/11/14 | 16/11/14 | 04/01/15 | 01/02/15 | 01/03/15 |
|---|---|---|---|---|---|---|
| IgG anti-HEV | + | | + | - | - | - |
| PCR HEV serum | | | + | + | - | - |
| PCR HEV stools | | + | | - | - | |
| AST (U/l) ALT (U/l) | 24<br>19 | | 26<br>17 | 28<br>16 | | |
| Bili tot. (µmol/l) | | | | 3 | | |
| PAL (U/l) | | | | | | |
| GGT (U/l) | | | 13 | | | |
| TP (%) | | | | 88 | | |

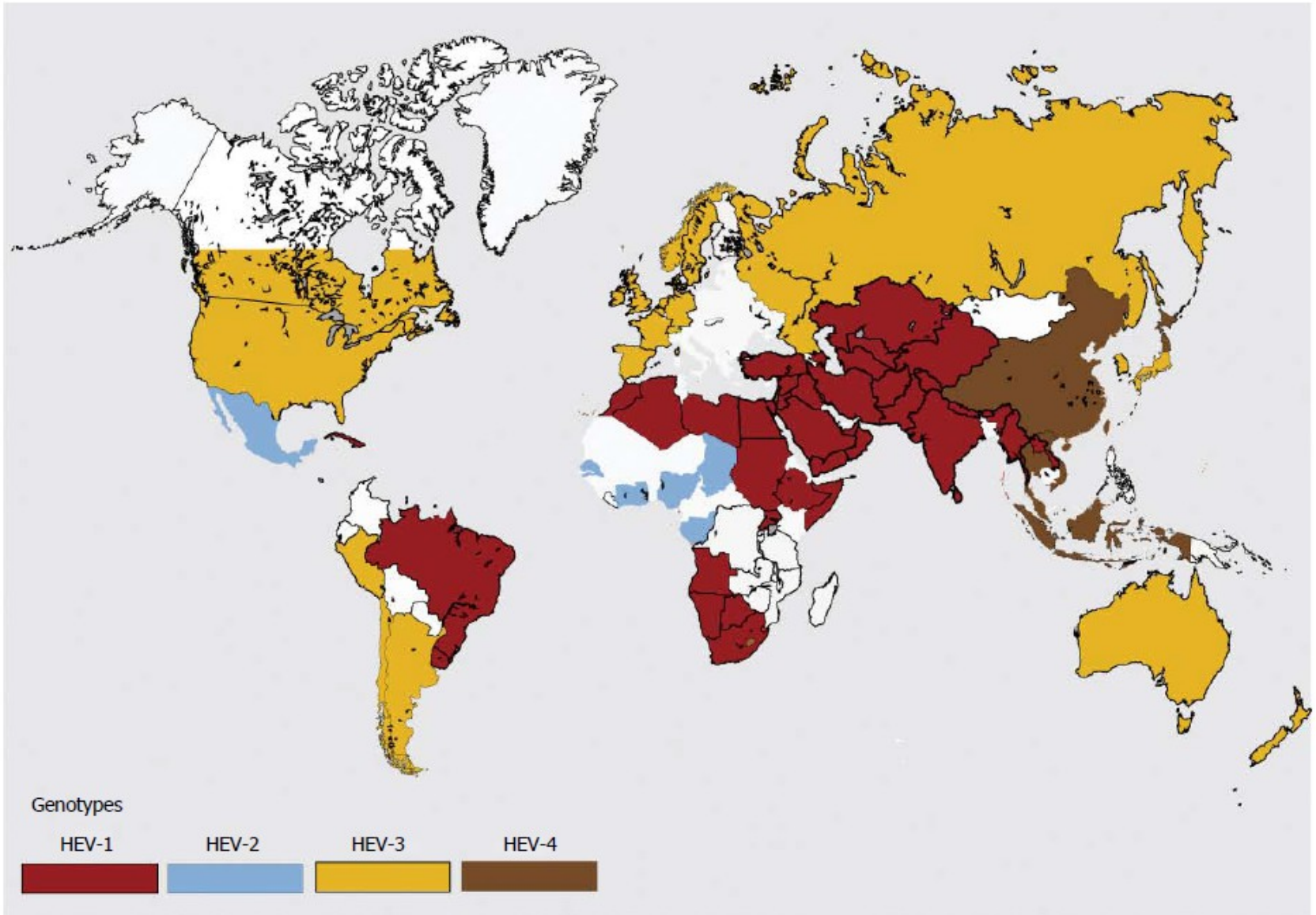
# Maden

| | 27/10/14 | 16/11/14 | 04/01/15 | 01/02/15 | 01/03/15 |
|---|---|---|---|---|---|
| IgG/M anti-HEV | - | - | - | | - |
| PCR HEV serum | | - | - | | - |
| PCR HEV stools | | | - | - | - |
| ASAT (U/l)<br>ALAT (U/l) | 47<br>27 | 29<br>21 | 39<br>22 | | |
| Bili tot. (µmol/l) | | | 3 | | |
| PAL (U/l) | | 221 | | | |
| GGT (U/l) | | 13 | | | |
| TP (%) | | | 97 | | |

# Have you any recommendation for the parents?

Scholar eviction?

- Family L mode of transmission?
  - No travel out of France
  - Shellfish consommation = (oysters + shrimps in Toulouse but also sausages….)
  - Swimming in the St Ferréol lake ?
  - + family history = (intrafamilial contamination with direct transmission via fecal-oral route ?)




Khuro et al, WJG 2016

# HEV GT 3 and 4: epidemiology

Endemic in some developing countries, as well as most high-income countries

Most common cause of acute viral hepatitis in many European countries

Estimated that ≥2 million locally acquired HEV infections/year

- Zoonotic infection
  - Primary hosts are pigs

HEV GT 3 and 4 tend to affect older males

- In an English study, male:female ratio was 3:1; median age, 63 years1

1. Dalton HR, et al. Eur J Gastroenterol Hepatol 2008;20:784–90;
EASL CPG HEV. J Hepatol 2018;doi: 10.1016/j.jhep.2018.03.005 [Epub ahead of print]

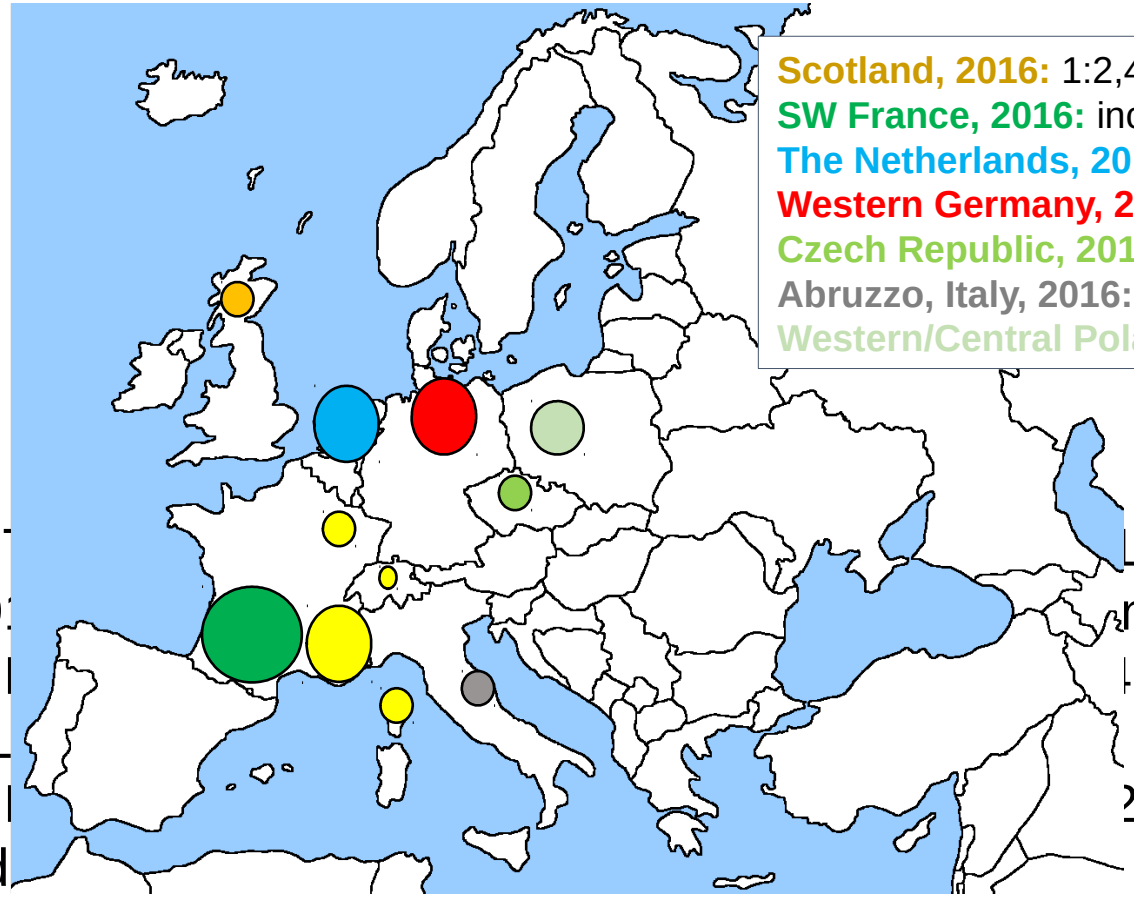
# HEV GT 3 and 4: epidemiology

Incidence varies between and within countries, and over time

- Multiple **'hotspots'** of HEV infection in Europe
- Even if zoonotic transmission has been identified as an important risk factor in high-come countries, the detection of HEV has been reported in several studies performed in environmental and irrigation water, sewage, and filter feeders.
- These results arise questions about the role of HEV in **other routes of transmission** and about its denomination as an environmental virus.

1. Dalton HR, et al. Eur J Gastroenterol Hepatol 2008;20:784–90;
EASL CPG HEV. J Hepatol 2018;doi: 10.1016/j.jhep.2018.03.005 [Epub ahead of print]

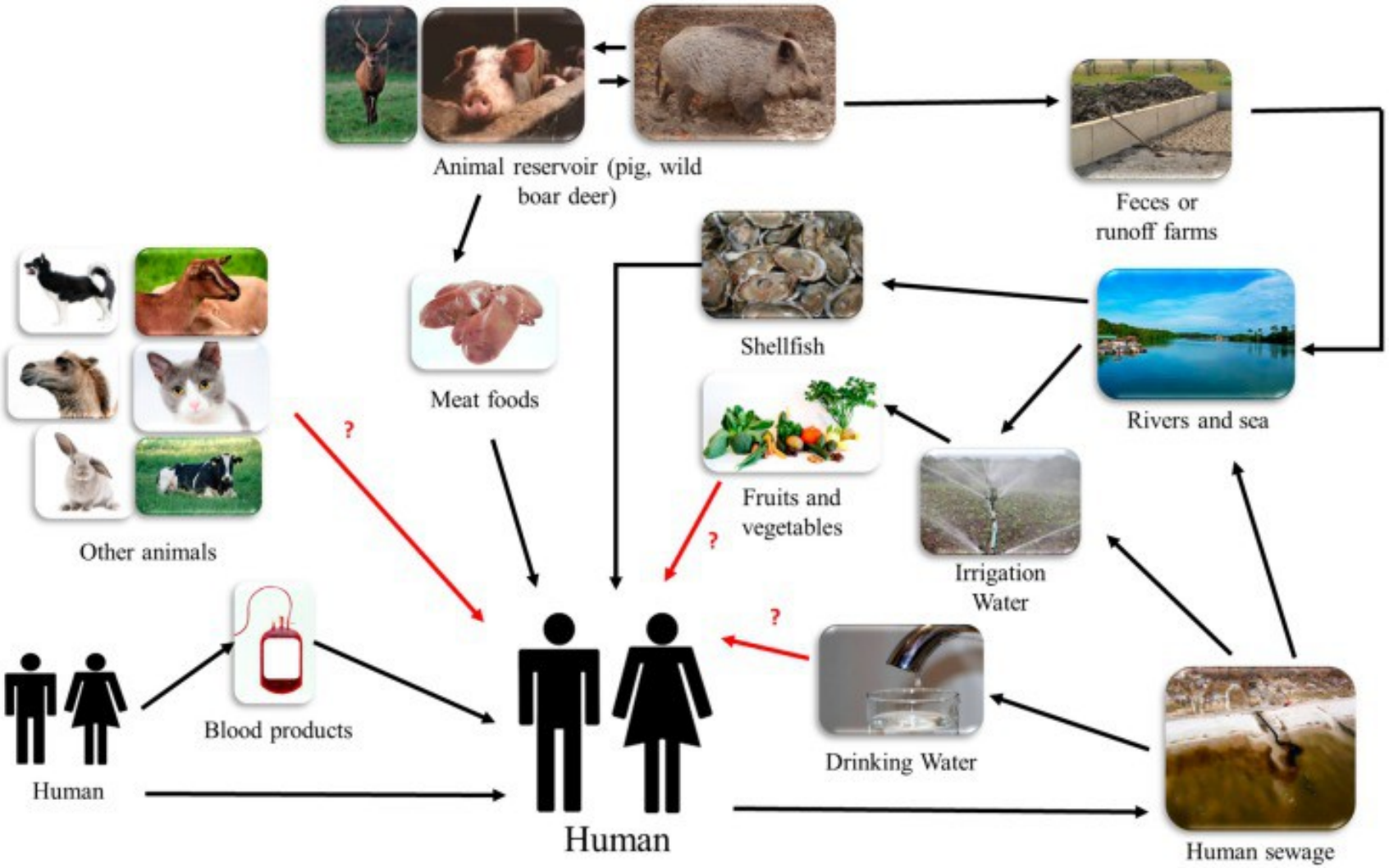
# HEV ‘hot spots’ in Europe



**Scotland, 2016:** 1:2,481 donors viraemic1
**SW France, 2016:** incidence 3–4%2
**The Netherlands, 2014:** 1:600 donors viraemic3
**Western Germany, 2015:** 1:616 donors viraemic4
**Czech Republic, 2015:** 400 lab-confirmed cases5
**Abruzzo, Italy, 2016:** seroprevalence 49%6
**Western/Central Poland, 2017:** seroprevalence 50%7


1. T 560-
79 nt];
2. I ; 3. Zaaijer
HL
4. I 2:1–66; 5.
Ad
6. Lucarelli C, et al. Euro Surveill 2016;21; 7. Bura M, et al, Int J Infect Dis. 2017; 61:20–2.
EASL CPG HEV. J Hepatol 2018;doi: 10.1016/j.jhep.2018.03.005 [Epub ahead of print]

- Modes of transmission of HEV in high income countries



# •HEV GT 1 and 2: epidemiology in developing countries or in travellers

- Epidemic and sporadic
- Fecal oral person to person contamination
- More then 20 Millions cases per year
- Young adults
- 20% symptomatic
- Severe forms : 70 000 deaths?
  - pregnant women (mortality : 20%)
  - Chronic liver diseases
- No chronic forms

*Rein, Hepatology 2O12*

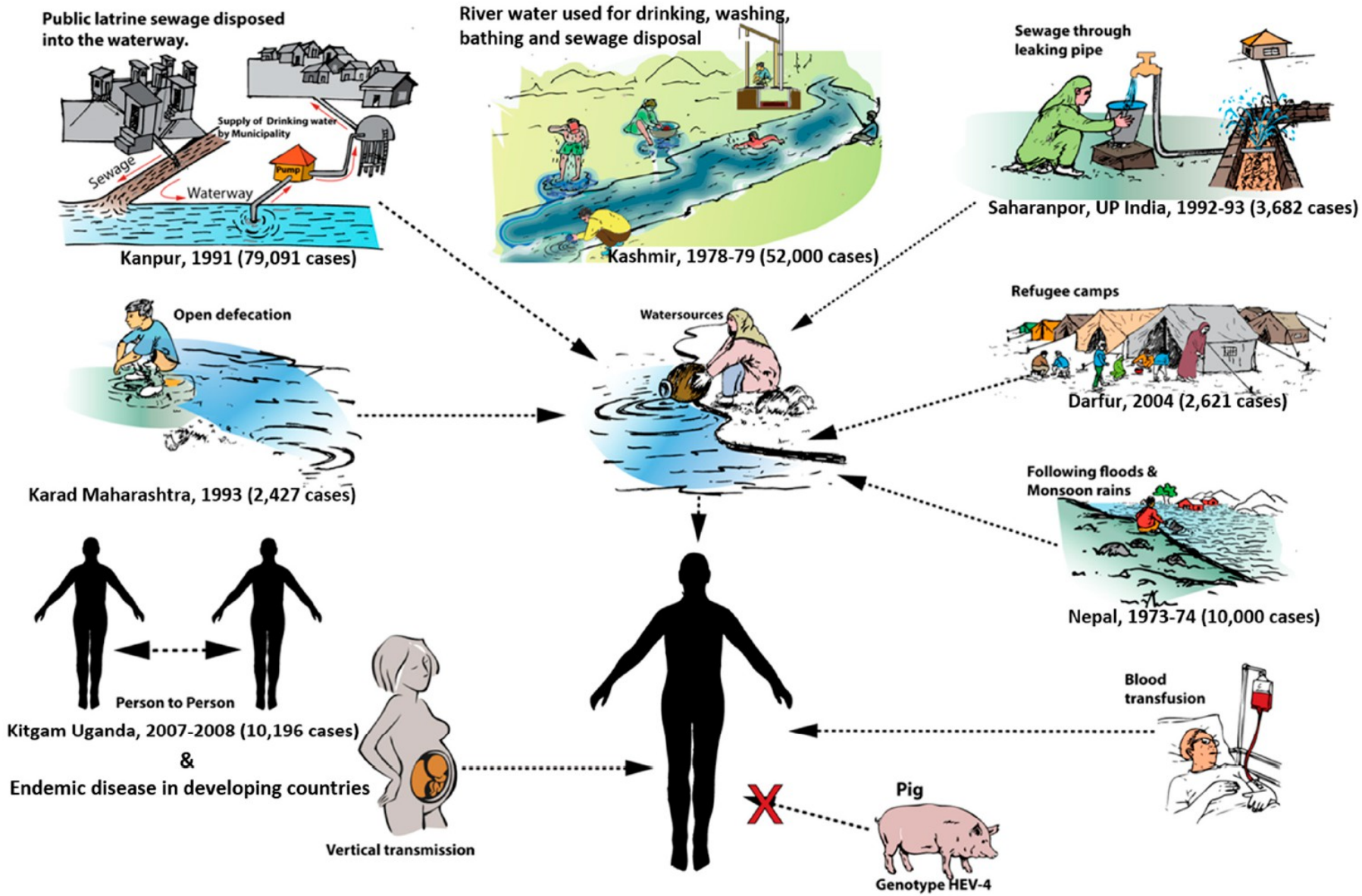



**Figure 2. Modes of transmission of hepatitis E in developing countries.** The settings for contamination of drinking water have been drawn in sketches, with epidemics reported in each case.


Thank
you !

# CONCLUSION : What Do We still Need to Know?

**ABOUT THE VIRUS** :Concerning the replication cycle of HEV, many points are still unknown, especially on the HEV receptor cell and on the exact role of the pORF3 protein. Cell culture progress and discoveries in these areas could lead to advances in treatment and vaccines.
The discovery of “quasi-enveloped” HEV particles in blood raises questions about the effects on infectivity, immunity, transmission, and the virus replication cycle.
**ABOUT THE EPIDEMIOLOGY** : The epidemiology of HEV in high-income countries is difficult to understand because of the small number of studies, the heterogeneity between them caused by the different assays used, and the type of population included. Gaps related to different suspected animal reservoirs, including food other than pork, need to be explored in order to improve the control and prevention of HEV.
**ABOUT THE CLINICAL PART** : the transition to chronicity and the real numbers of symptomatic cases are still poorly understood. Studies could be conducted to deepen knowledge.

# ABOUT PREVENTION

Despite the obvious risk of HEV transmission, prevention strategies are currently insufficient and infrequently implemented.

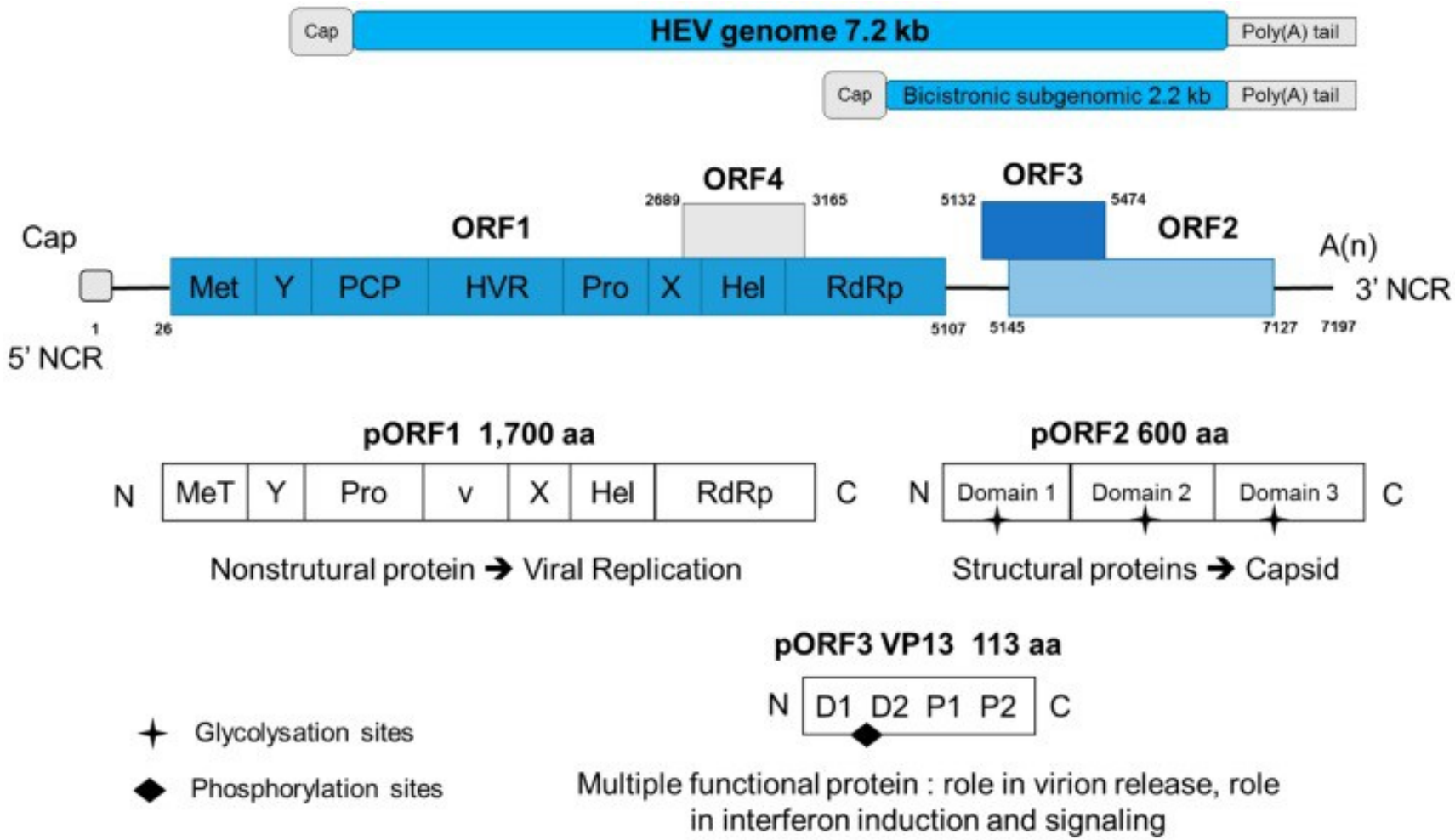
Hepatitis E remains unknown to the general public. The population could be sensitized, particularly in the hyperendemic regions. Risky behaviors may thus decrease, especially among people at risk.

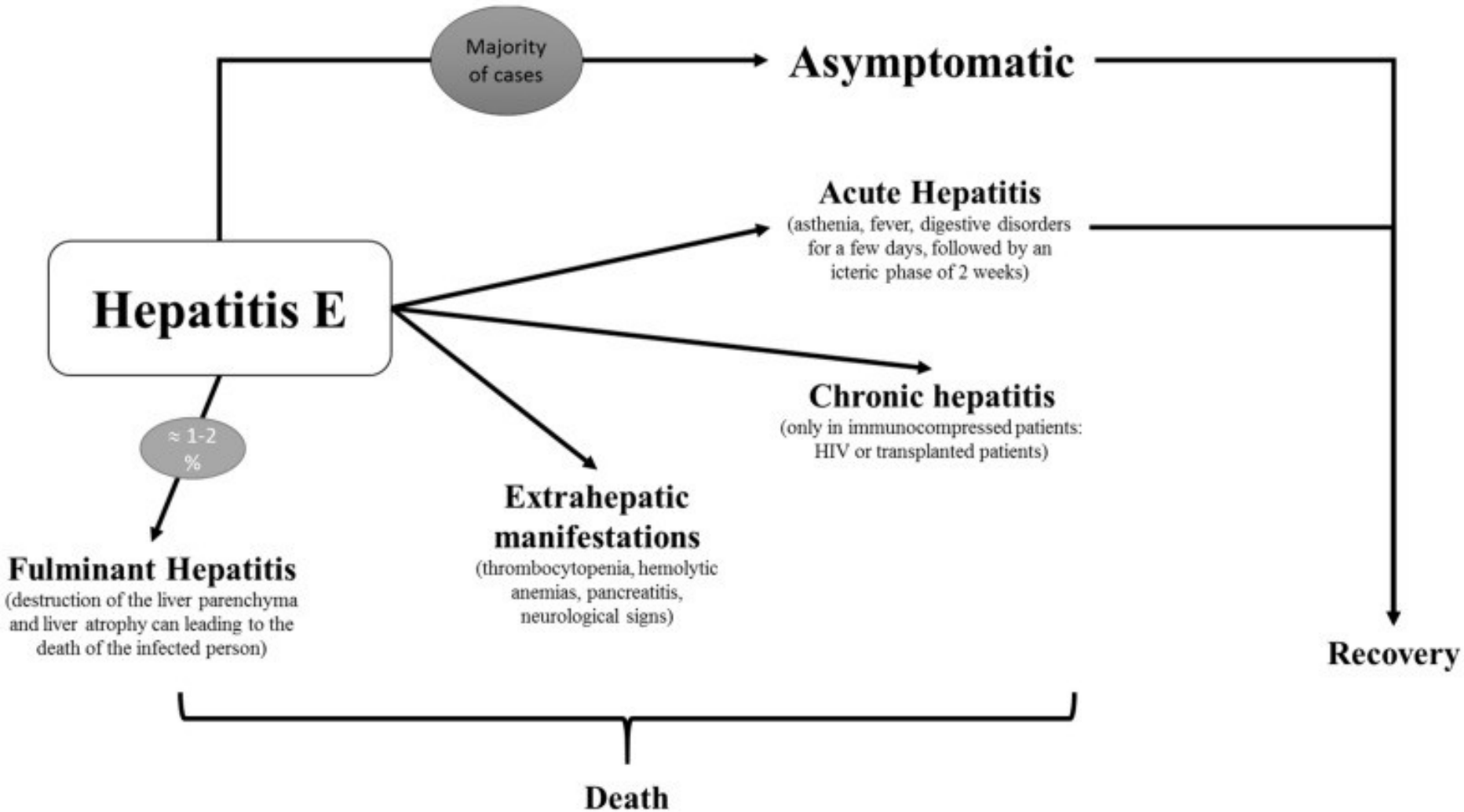
Several studies have been undertaken to develop a human vaccine against HEV. A HEV vaccine is currently available in China*, but not in other countries worldwide.

No vaccines are currently available in industrialized countries where HEV transmission is predominantly zoonotic;

The development of a vaccine in pigs could reduce the spread of the virus between species and humans.

**A study undertaken with more than 50,000 participants showed a vaccine efficacy of 100% after 12 months and 86.8% (95% confidence interval 715–945) after a prolonged follow-up of 4.5 years . People who received three doses (0, one, and six months) maintained their antibodies against HEV for at least 4.5 years. Currently, this vaccine against HEV has obtained approval from the Chinese government*


Cap
HEV genome 7.2 kb
Poly(A) tail
Cap
Bicistronic subgenomic 2.2 kb
Poly(A) tail
ORF4
2689
3165
ORF3
5132
5474
ORF1
ORF2
Cap
A(n)
Met
Y
PCP
HVR
Pro
X
Hel
RdRp
3' NCR
1
26
5107
5145
7127
7197
5' NCR
pORF1 1,700 aa
N
MeT
Y
Pro
v
X
Hel
RdRp
C
Nonstrutural protein ➔ Viral Replication
pORF2 600 aa
N
Domain 1
Domain 2
Domain 3
C
Structural proteins ➔ Capsid
pORF3 VP13 113 aa
N
D1
D2
P1
P2
C
Glycolysation sites
Phosphorylation sites
Multiple functional protein : role in virion release, role in interferon induction and signaling


Majority of cases
Asymptomatic
Acute Hepatitis
(asthenia, fever, digestive disorders for a few days, followed by an icteric phase of 2 weeks)
Hepatitis E
Chronic hepatitis
(only in immunocompressed patients: HIV or transplanted patients)
≈ 1-2 %
Extrahepatic manifestations
(thrombocytopenia, hemolytic anemias, pancreatitis, neurological signs)
Fulminant Hepatitis
(destruction of the liver parenchyma and liver atrophy can leading to the death of the infected person)
Recovery
Death

# Pathogenesis

P

## Asymptomatic

- 2/3 cases
- Cholestatic forms????

## Acute Hepatitis :

- Mortality rate : 0,2 to 4 %
- higher :
  - Pregnant women: 10 to 25 %
  - Chronic liver disease: 70 % mortality at 1 year(3) Kumar 2007

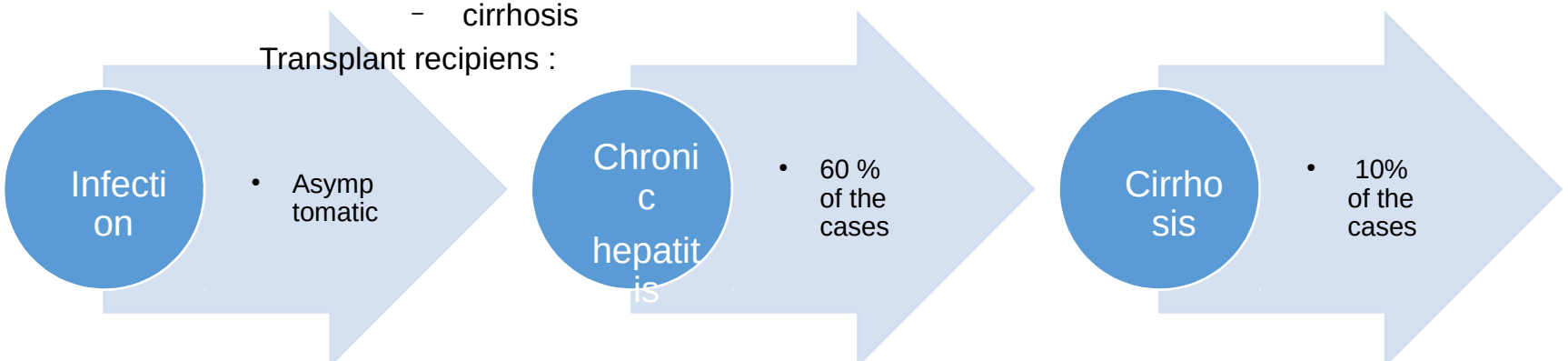
## Chronic hepatitis in immunosuppressed patients(4) (5) Abrava

- Genotype 3
- ALT elevation
- Diagnostic : PCR +++
- cirrhosis

Transplant recipiens :

| Symptômes | % |
|---|---|
| Jaunisse | 67.7 |
| Asthénie | 40.3 |
| Fièvre | 27.4 |
| Arthromyalgie | 21 |
| Douleur abdominale | 11.3 |
| Céphalée | 9.7 |
| Nausée | 9.7 |
| Anorexie | 8.1 |
| Perte de poids | 6.5 |
| Diarrhée | 4.8 |
| Eruption | 3.2 |

are unknown. (E) Miscellaneous clinical syndromes (eg, HEV3 has been associated with glomerulonephritis).[95]



# Pathogenesis

## Extrahepatic Manifestations

- Neurological : >10% (5) Kamar 2011
- Others : thrombopenia (6) Colson 2008, pancreatitis (7) Deniel 2011, nephropathies (8) Ali 2001 (9) Kamar 2012

## Seroprevalence :

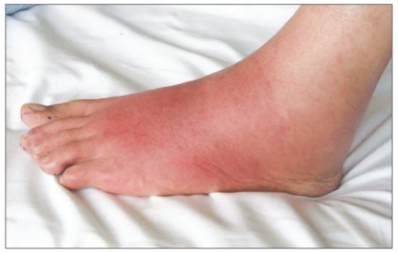
- Developing countries: 1 to 76 %
- High income countries: variable
- Tests sensitivity!!!!

(11) Mansuy 2011

# Extrahepatic Manifestations

✓ Glomerulonephritis ± cryoglubulinemia

Kamar, *AJKD* 05 ; Kamar, *Am J Transplant* 11 ; Kamar, *Transplantation* 12, Pishke, *Lancet Infect Dis* 15; Guinaut, Am J Kidney Dis, 16



✓ Thrombopenia, acute pancreatitis

Colson, *J Clin Microbiol* 08 ; Fouquet, *J Clin Virol* 10 ; Deniel, *J Clin Virol* 11

✓ Neurological signs ~ 15%

➔ Guillain-Barré, Parsonage-Turner, meningo-encephalitis, multinevritis, …

Kamar, *Am J Transplant* 10 ; Kamar, *Emerg Infect Dis* 11 ;
Maurissen, *Infection* 11 ; Despierres, *Emerg Infect Dis* 11 ;
Colson, *Clin Res Hepatol* 13 ; Geursvankessel, *Clin Infect Dis* 13 ;
van den Berg, *Neurology* 14 ; van Eijk, *Neurology* 14…

# HEV and neurology

464 patients (neurologic emergencies)
(without trauma)

11 recent acute infections
Parsonage Turner n=3
AVC n=4
Encephalitis n=1
Epilepsy n=2
nerve VII paralysis n=1

Median ALT =24 (8-145)
No icterus

152 resolved or passed Infections (32,7%)

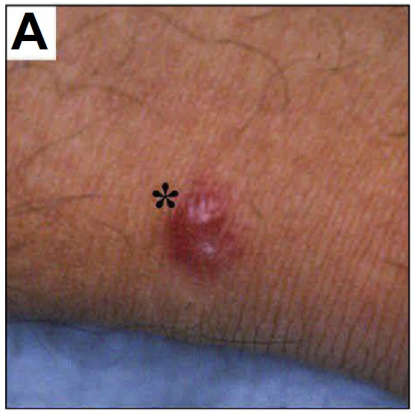
IgG+, IgM et PCR -

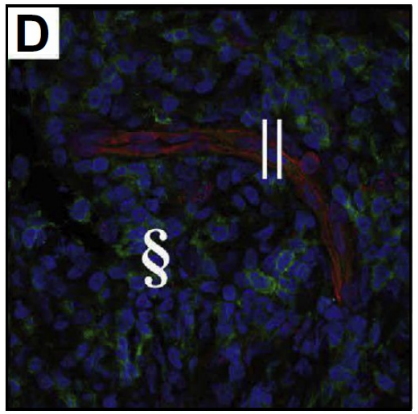
301 without Infection

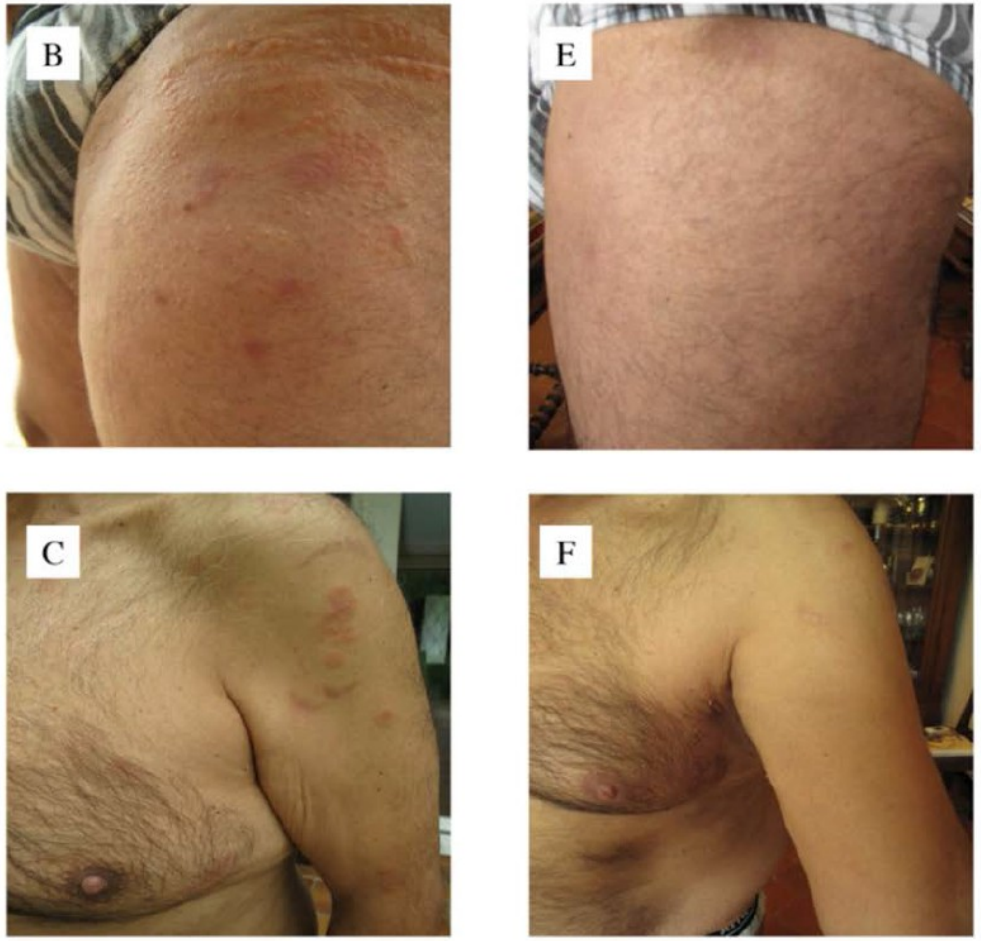
IgG, IgM and PCR -

Dalton, *J Hepatol 2017*

# HEV and lymphoproliferative disorders




Regression after anti-viral treatment (IFN + RBV)

Mallet, *J Hepatol 2017*

# Chronic Infection in immunosupressed patients

- Transplanted Patients (solid organs)

  Kamar, *NEJM 2008 ;* Gerolami, *NEJM 2008 ;*
  Haagsma, *Liver Transpl 2008 ;* Halac, *Gut 2011*

- Hemato-oncology Patients Peron, *J Gastroenterol Hepatol 2006* ;
  Tamura, *Hepatol Res 2007* ; Ollier, *Ann inter Med 2009*
  Mansuy, *Clin Infect Dis* 2009 ; Tavitian, *J Clin Virol* 2010; *Emerg Infect Dis* 2015

- Infected Patients and CD4 < 200/mm3

  Dalton, *NEJM* 2009 ; Colson, *J Clin Virol* 2009 ; Kenfak, *Emerg Infect Dis* 2011;
  Kuniholm, *Hepatology* 2015

- Patients under immuno-therapy ?

- Ecology of HEV

  - Human reservoir: principal HEV reservoir in developing countries (genotype 1 (persistance and virus excretion in stools)
  - Animal reservoir : important = primates, porcs, chicken etc, … → zoonosis in high income countries

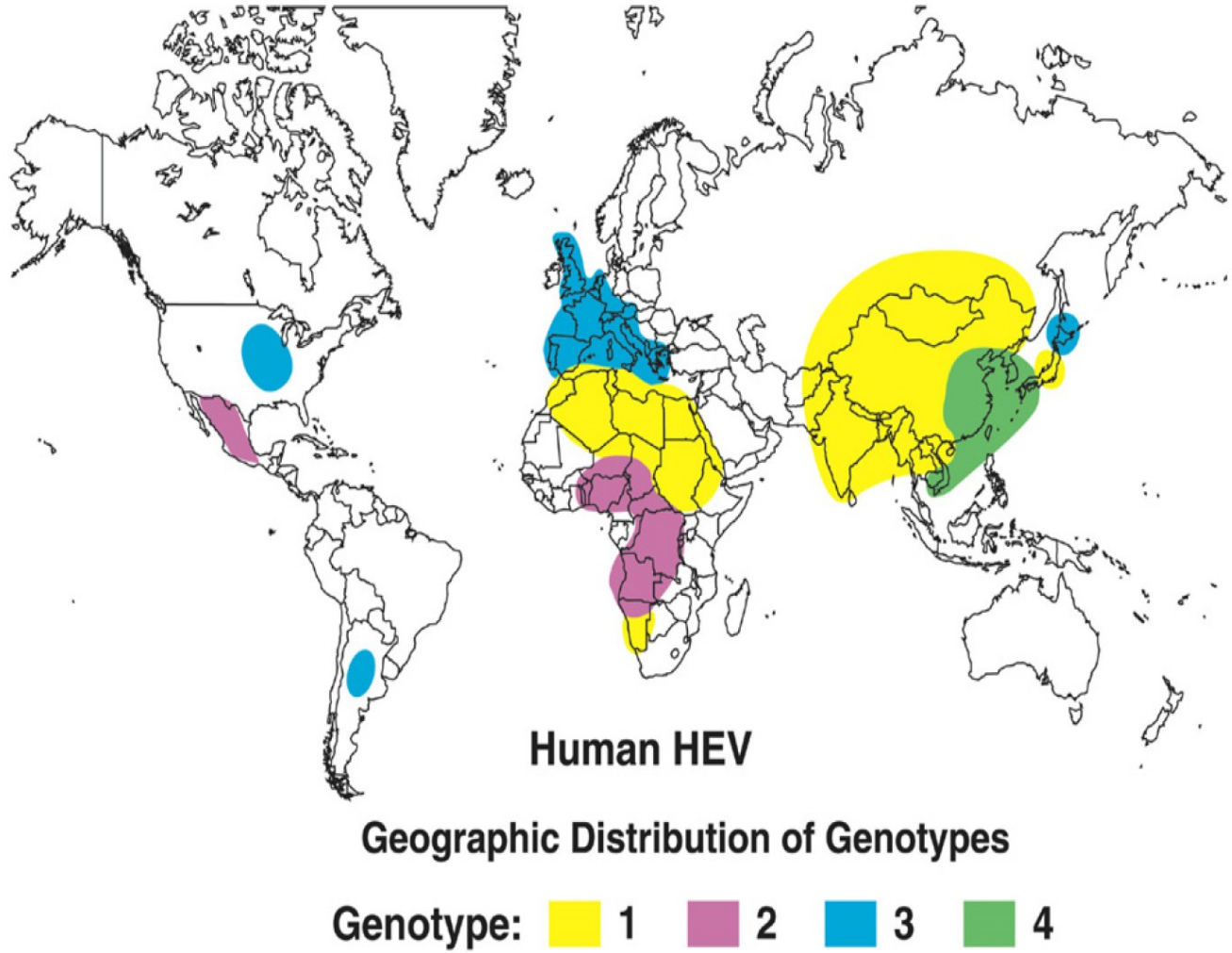



Fig. 4. Each of the fo HEV that infect humans has a distinct, and in some cases, overlapping geographic distribution.