HEV RISK ASSESSMENT FOR PLASMA PRODUCTS

Dr Benoît FLAN, Pharm D, PhD
Director of Biological Safety Surveillance
INTRODUCTION

● Proven HEV transfusion risk (France, Japan, UK, Germany...)
  ▪  +20 notifications in France (ANSM)
  ▪  **12 Post – transfusion notifications (HEV RNA +ve Plasma) received at LFB**
    ➡️ 11 donations fractionated; 3 on stock (discarded)

● Confirmed high seroprevalence and incidence in France (geno. 3)
  ▪  endemic (hyper- in some areas): 22,4 % (8-86,4 %) *(Mansuy, Hepatology 2016)*
  ▪  1 HEV RNA +ve / 2,218 donations *(Gallian, EID 2014)*
  ▪  **5 Look-back information from SD plasma HEV RNA testing**
    ➡️ 4 donations fractionated; 1 on stock (discarded)

● No transmission documented from plasma derivatives
  ▪  risk assessment for PDMP
ASSESSING THE RISK FOR VIRUS TRANSMISSION

- Guideline on plasma-derived medicinal products
  EMA/CHMP/706271/2010 - Chapter 9

  - ...where possible, include a quantitative estimation of the probability of a virus contaminant being present in a defined dose of final product

  - “overall virus inactivation/removal capacity” of the mfg process
    - CPMP guideline on virus validation (CPMP/BWP/268/95)
    - for emerging viruses:
      - relevance of model viruses (ie HAV 27 – 30 nm for 15/20 N Nanofiltration)
      - investigational studies recommended

  - “potential virus input”: potential amount of virus that may be present in the amount of starting material needed to manufacture a single dose of product
    - number of viraemic donations in the mfg pool, volume of individual donations, titre of a viraemic donation
    - amount of plasma for production of one vial of product
● Introduction

● Parameters of Risk assessment for PDMP
  ▪ Exposure: Viremia in HEV RNA +ve donations ➔ IU/ml
  ▪ Reduction: virus Inactivation / Elimination steps ➔ Infectivity
    o HEV infectivity assay
    o Nanofiltration 35 nm
    o Efficacy of VI/VE steps (summary)
  ▪ Correspondance between HEV RNA and Infectivity
    o Towards definition of a *minimum infectious dose* for Risk assessment of PDMP
VIREMIA IN HEV RNA +VE DONATIONS

- low to moderate viremia in HEV +ve donations

(from Gallian, EID 2014)
OUTLINE

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HEV INFECTIVITY ASSAY
(LHOMME et al, J.VIROL 2014)

VIROLOGY Laboratory, CH TOULOUSE, FRANCE, Pr J. IZOPET

- HEV (genotype 3f) sources:
  - faecal sample from acute HE patient
  - cell supernatant
- HEV replicating Cells: Hep G2/C3A (ATCC)
- Virus stock: $10^8 – 10^{10}$ HEV RNA copies/ml - ~ 5 log TCID$_{50}$ / ml
  - HEV RNA/TCID$_{50}$ Ratio = 4,28 log copies +/- 0,91 (n=7)
- Detection and quantification of HEV RNA
  - in house RT-PCR (Abravanel, J Clin Microbiol 2012)
- Titration
  - Infectivity Assay (TCID$_{50}$/ml)
    - $de$ $novo$ HEV RNA (RT-PCR) in cell culture
    - infectivity titration (+ve wells): Reed et Muench
  - Q-PCR (nanofiltration experiments)
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HEV ~ ENVELOPED VIRUS: IMPACT ON SIZE AND SERONEUTRALIZATION

- HEV in feces and bile: non enveloped
- In circulating blood and culture supernatant, HEV covered with a cellular membrane similar to EV
- Estimated Size (TEM)
  - membrane associated HEV particles ~50 nm (secretion associated with exosomes)
  - without outer membrane: 30 – 35 nm diameter
- Buoyant density (gradient)
  - HEV in serum (and culture): 1,15 - 1,16 g/ml
  - HEV in faeces: 1,27 – 1,28 g/ml
- HEV in serum non neutralizable by immune sera the difference to HEV from feces


PLC/PRF/5 (a), HepG2 (b) and A549 (c) cells infected with cell culture-produced HEV (JE03-1760F strain). Arrows = extracellular membrane-associated virus-like particles. Scale bars: 50nm.
35 N NANOFILTRATION OF VWF

Study Design

Spiking (1%) of VWF solution (0.2 µm pre-filtered)

0.1 µm pre-filtration

35nm Nanofiltration

Load

Filtrate

Analysis of samples

sample → storage

RT-qPCR

Inoculation (Hep G2c3a)

Endpoint dilution 6 replicates/dil.

Washes

RT-qPCR

% Pos. wells

Day 0

Day 1

Day +4
## HEV REDUCTION – NANOFILTRATION PLANOMA 35N

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>Von Willebrand Factor (VWF)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Virus (treatment)</strong></td>
<td>HEV Cell Supernatant (+ Detergent - NP40)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viral load (log TCID&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>RNA copies (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spike</strong></td>
<td>5,4</td>
</tr>
<tr>
<td><strong>Load (0.1 µm)</strong></td>
<td>5,3</td>
</tr>
<tr>
<td><em>(Load 2 – after low pH)</em></td>
<td>-</td>
</tr>
<tr>
<td><strong>35 N Filtrate</strong></td>
<td>2,8</td>
</tr>
<tr>
<td><strong>RF</strong></td>
<td><strong>2.5</strong></td>
</tr>
<tr>
<td></td>
<td><strong>2,1</strong></td>
</tr>
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### HEV REDUCTION – NANOFILTRATION PLANOVA 35N

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>Von Willebrand Factor (VWF)</th>
<th>VWF</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus (treatment)</td>
<td>HEV Cell Supernatant (+ Detergent - NP40)</td>
<td>HAV (+ CHCl3)</td>
<td>HEV Cell Supernatant (+ EtOH 22%)</td>
</tr>
</tbody>
</table>

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<th>RNA copies (log)</th>
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</thead>
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<tr>
<td>Spike</td>
<td>5.4</td>
<td>9.5</td>
<td>7.4 (load 1)</td>
<td>9.4</td>
</tr>
<tr>
<td>Load (0.1 µm)</td>
<td>5.3</td>
<td>9.3</td>
<td>7.4</td>
<td>9.5</td>
</tr>
<tr>
<td>(Load 2 – after low pH)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.2</td>
</tr>
<tr>
<td>35 N Filtrate</td>
<td>2.8</td>
<td>7.2</td>
<td>7.0</td>
<td>6.1*</td>
</tr>
<tr>
<td>RF</td>
<td>2.5</td>
<td>2.1</td>
<td>0.4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*free RNA accdg to RNase digestion studies

- Significant reduction of infectivity of HEV through 35 nm nanofiltration of VWF
- HEV reduction on 35 nm filter > HAV (~ no reduction)
- (at least) 3.1 log HEV RNA copies reduction through 35 nm nanofiltration of IgG
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HEV TRANSFUSION CASES (SUMMARY 1/2)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Component</th>
<th>Volume (ml plasma)</th>
<th>HEV RNA (log IU/ml)</th>
<th>HEV RNA (total – log IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsui, Hepatol Res 2014</td>
<td>Japan</td>
<td>Platelets</td>
<td>-</td>
<td>-</td>
<td>6.8</td>
</tr>
<tr>
<td>Hauser, Blood 2014</td>
<td>France</td>
<td>FFP</td>
<td>Min. 200</td>
<td>? (estimate 100 IU/ml)</td>
<td>? (&gt; 4.3)</td>
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<tr>
<td>Haïm-Boukobza, J Hepatol 2012</td>
<td>France</td>
<td>Platelets</td>
<td>(estimate 3 – 30)</td>
<td>4.2</td>
<td>~ 5</td>
</tr>
<tr>
<td>Matsubayashi, Transfusion 2008</td>
<td>Japan</td>
<td>Platelets</td>
<td>-</td>
<td>-</td>
<td>5.4</td>
</tr>
<tr>
<td>Colson, EID 2007</td>
<td>France</td>
<td>RBCU</td>
<td>310</td>
<td>? (estimate 100 IU/ml)</td>
<td>? (&gt; 4.5 *)</td>
</tr>
</tbody>
</table>

*data from literature
## HEV TRANSFUSION CASES (SUMMARY 2/2)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Component</th>
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<th>HEV RNA (total – log IU)</th>
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</thead>
<tbody>
<tr>
<td>Huzly, Eurosurv 2014</td>
<td>Germany</td>
<td>Platelets (apheresis)</td>
<td>196, 247</td>
<td>2.1, 3.95</td>
<td>3.85, 3.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platelets (apheresis)</td>
<td>No transmission</td>
<td>2.7</td>
<td>-</td>
</tr>
<tr>
<td>Hewitt, Lancet 2014</td>
<td>UK</td>
<td>RBC, Platelets, Plasma</td>
<td>4.53</td>
<td>&gt; 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No transmission</td>
<td>2.57</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Boxall, Transfus Med 2006</td>
<td>UK</td>
<td>RBCU</td>
<td>Transmission 30 mL</td>
<td>? (estimate 100 IU/mL)</td>
<td>? (&gt; 3,5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platelets</td>
<td>No transmission</td>
<td>? (estimate 100 IU/mL)</td>
<td>-</td>
</tr>
<tr>
<td>Matsubayashi, Transfusion 2004</td>
<td>Japan</td>
<td>FFP</td>
<td>Transmission 200-300 mL</td>
<td>? (estimate 100 IU/mL)</td>
<td>? (&gt; 4.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RBCU</td>
<td>No transmission</td>
<td>? (estimate 100 IU/mL)</td>
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<tr>
<td>Hewitt, Lancet 2014</td>
<td>UK</td>
<td>RBC, Platelets, Plasma</td>
<td>Transmission (min 3mL)</td>
<td>4.53</td>
<td>&gt; 5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>min 3 log</td>
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<td>? (&gt; 3.5)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platelets</td>
<td>No transmission 3 - 4 mL</td>
<td>? (estimate 100 IU/mL)</td>
<td>-</td>
<td>min 2.7 log</td>
</tr>
<tr>
<td>Matsubayashi, Transfusion 2004</td>
<td>Japan</td>
<td>FFP</td>
<td>Transmission 200-300 mL ?</td>
<td>? (estimate 100 IU/mL)</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RBCU</td>
<td>No transmission 30-100 mL ?</td>
<td>? (estimate 100 IU/mL)</td>
<td>-</td>
<td>min 3.5 log</td>
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*data from literature*
Data from 22 transfusion HEV transmission cases in France
(19 cases from ANSM, 08/2006 – 07/2014, updated from LFB data)

- Imputability: 14/19 certain, 5/19 probable
- All blood components involved: Plasma (S/D plasma, quarantined FFP, AI FFP), RBC, Platelets (pooled Platelets concentrates, apheresis platelets)
- HEV RNA Quantification: 20 (?*) – 2.5 × 10⁵ copies/ml
- Considering minimal plasma volume per component (worst case)
  - Total HEV RNA content in donations which transmitted
    - 20/22 cases > 3.9 log IU (11/22 > 5 log)
    - 2 (probable) cases: 3.16 (?*) - > 3.45 log IU

* genotype divergence
SUMMARY AND CONCLUSION

● No evidence to date that HEV represents a risk to PDMP: no history of residual hepatitis risk through the use of PDMP

● Historical HEV circulation in developed countries with high seroprevalence and incidence in donor populations

● Risk assessment for PDMP (PTC)
  - low to moderate viremia in HEV RNA +ve donations
  - efficient steps towards NEV in PDMP manufacturing processes
  - available data from transfusion cases favour a 4 log HEV RNA correspondence to Infectivity which should be considered in the HEV risk assessment model for PDMP
ACKNOWLEDGEMENTS

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  - Pr Jacques IZOPET
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THANK YOU...
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