Molecular Epidemiology of HCV infections in the Netherlands.

Thijs van de Laar
GGD Amsterdam
Molecular Epidemiology of HCV

- HCV - genetic variability:
  - 7 genotypes – 30-35%
  - 89 subtypes – 15-30%

- Genetic diversity:
  - Geographic origin
  - Mode of transmission
  - Timescale of spread

- To compare we need:
  - Consensus region (NS5B)
  - Unified classification system

Molecular Epidemiology

- General population
- Injecting drug users (IDU)
- Men who have sex with men (MSM)
General population – HCV prevalence

- Estimated HCV prevalence (0.1-0.4%)

<table>
<thead>
<tr>
<th>Population</th>
<th>Year</th>
<th>Nr screened</th>
<th>Prevalence</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch population</td>
<td>1995</td>
<td>7373</td>
<td>0.08%</td>
<td>Veldhuijzen</td>
</tr>
<tr>
<td>A’dam pregnant*</td>
<td>2003</td>
<td>5146</td>
<td>0.31%</td>
<td>Urbanus</td>
</tr>
<tr>
<td>A’dam population</td>
<td>2005</td>
<td>1355</td>
<td>0.66%</td>
<td>Baaten</td>
</tr>
<tr>
<td>Arhem/Nijmegen</td>
<td>2006</td>
<td>2200</td>
<td>0.18%</td>
<td>Slavenburg</td>
</tr>
</tbody>
</table>

* Testing still ongoing
HCV genotype distribution the Netherlands:

- **GT 1a/b**: 50% difficult (50%)
- **GT 2a/b**: 10% good (90%)
- **GT 3a**: 30% good (90%)
- **GT 4a/d**: 10% intermediate (60%)

Prevalent HCV infections BUT genotype distribution is dynamic

- **N=179 Lanl**
- **N=81 Blood bank Van de Laar et al, 2005**
- **N=351 Hospital De Vries et al., 2006**
Prevalent HCV infection – general population

- **Epidemiological profiles** of HCV prevalent candidate donors

<table>
<thead>
<tr>
<th>Transmission route</th>
<th>%</th>
<th>Genotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood products before 1991</td>
<td>30%</td>
<td>1b + 2a/b + (1a)*</td>
</tr>
<tr>
<td>IDU-related</td>
<td>21%</td>
<td>3a + 1a + 4d</td>
</tr>
<tr>
<td>Endemic countries</td>
<td>12%</td>
<td>4a + ‘Other’</td>
</tr>
<tr>
<td>Other parenteral</td>
<td>19%</td>
<td>mix</td>
</tr>
<tr>
<td>No risk</td>
<td>18%</td>
<td>1b + 2a/b</td>
</tr>
</tbody>
</table>

*Incidental spillover from high risk groups to the general population

*Van de Laar et al. (2006) Transfusion*
Incident HCV infection – general population

<table>
<thead>
<tr>
<th>Transmission route</th>
<th>Patient</th>
<th>Genotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupational/Health care</td>
<td>F, 35 yrs</td>
<td>1b</td>
</tr>
<tr>
<td>Nosocomial/Hospitalisation (B)</td>
<td>M, 55 yrs</td>
<td>Untyped</td>
</tr>
<tr>
<td>Sexual/household</td>
<td>F, 42 yrs</td>
<td>3a (partner ex-IDU)</td>
</tr>
<tr>
<td>Sexual/household</td>
<td>F, 56 yrs</td>
<td>3a (partner ex-IDU)</td>
</tr>
<tr>
<td>Sexual/household</td>
<td>F, 40 yrs</td>
<td>RNA-negative</td>
</tr>
</tbody>
</table>

* Incidental spillover (from high risk groups) to the general population through mostly non-parenteral modes of transmission

Van de Laar et al. (2006) Transfusion
Spread general population

- Screening of donorblood (1992):
  - Donor selection
  - HCV serology and HCV-RNA

  - 1:31.500.000 donations (negligle)

- Incidental spillover high-risk populations
  Ongoing spread in dialysis centres (1995-1997)

Consequence: Genotype shift towards less HCV GT 1b and 2a/b

Injecting drug users (IDU)

- HCV prevalence (50-80%)
- IDU genotypes: 1a and 3a
  - 1a: 3 separate introductions
  - 3a: 1 single introduction

Timescale - spread of HCV in Europe

Two-phase epidemic:

- **Steady state**: west African origin
- **Exponential growth**: 1940 IDU

Injecting drug users (1985-2005)

- Less injection drug use: impopularity
  - 1985-1989: 90% DU < 30 yrs ever injected
  - 2000-2004: 31% DU < 30 yrs ever injected
  HCV prevalence young DU: 83% → 14%

- Safer injection: Harm reduction
  - NEP combined with methadone treatment
  - Larger window of intervention
  HCV prevalence young IDU: 91% → 44% (30%)

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Molecular epidemiology of HCV

HCV strains young IDU Amsterdam

- **Genotype 1a(II)**
- **Subclade 1a(I)**
- **1985-1989**
- **2000-2004**

**INTRODUCTION 4d**

HCV strains young IDU Amsterdam

1985-1989

2000-2004

Genotype 1a(II)

Subclade 1a(I)

Genotype 3a
Injecting drug users (IDU)

- Diversification of HCV genotype 1a and 3a
- Introduction and spread of HCV genotype 4d
- Genotypic shift towards difficult-to-treat genotypes (1 and 4)
- HCV incidence is low → TREATMENT to decrease HCV reservoir further
HCV treatment of IDU

Cost-effective?

What about HCV reinfection and superinfection?
HCV reinfection and superinfection

- **Reinfection – protective immunity**
  - **YES:** Incidence of HCV reinfection < Incidence initial infection
  - **NO:** Incidence HCV reinfection = incidence initial infection

- **Amsterdam HCV seroconverter (SC) study**
  - 59 HCV seroconverters (1985-2005)
  - 5 time points per SC (RNA + viral typing)
  - Frequency HCV reinfection and superinfection

Results: SC without viral clearance
Reinfection / Superinfection

- MULTIPLE HCV infections (41%)
- 1-4 HCV strains per IDU
- Reinfection with same HCV subtype
- Lack of immunological protection
New risk groups: MSM

- Since 2000: Increased HCV-incidence among MSM in the UK.

- More case reports:
  - The Netherlands (Ruijs 2004; Agtmael 2004; Götz 2005)
  - France (Ghosn 2004; Gambotti 2005)
  - Switzerland (Rauch 2005)
  - Germany (Vogel 2005)
  - England (Gilleece 2005)
  - Australia (Matthews, 2006)
  - United States (Bateman, 2006)

HCV incidentie MSM in NL

- Screening Amsterdam Cohort Studies
  - 1985-1999: 0.8/1000 PY
  - 2000-2002: 8.7/1000 PY

- Hospital notifications: 2002-2005 (n=34)
  - High risk sexual behavior
  - HIV-positive (97%)
  - Genotypes 1 en 4 (92%)

Outbreak:
MSM-specific HCV strains

van de Laar et al (2007) JID
England: genotype 1a

France: genotype 4d

Danta et al (2001) AIDS

Sexual HCV transmission

Incidental national outbreaks

or

large international transmission network?
### Clustering (85% of MSM)

<table>
<thead>
<tr>
<th>Clusters en Paren</th>
<th>Genotype</th>
<th>Country mixing</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1 n = 37</td>
<td>1a</td>
<td>yes</td>
<td>UK, NL</td>
</tr>
<tr>
<td>Cluster 2 n = 34</td>
<td>4d</td>
<td>yes</td>
<td>UK, NL, D, F</td>
</tr>
<tr>
<td>Cluster 3 n = 19</td>
<td>1a</td>
<td>yes</td>
<td>UK, NL, D</td>
</tr>
<tr>
<td>Cluster 4 n = 17</td>
<td>1a</td>
<td>yes</td>
<td>UK, D</td>
</tr>
<tr>
<td>Cluster 5 n = 12</td>
<td>1a</td>
<td>yes</td>
<td>UK, NL, D, AUS</td>
</tr>
<tr>
<td>Cluster 6 n = 12</td>
<td>1a</td>
<td>no</td>
<td>UK</td>
</tr>
<tr>
<td>Cluster 7 n = 6</td>
<td>1a</td>
<td>yes</td>
<td>NL, D</td>
</tr>
<tr>
<td>Cluster 8 n = 6</td>
<td>1a</td>
<td>no</td>
<td>AUS</td>
</tr>
<tr>
<td>Cluster 9 n = 6</td>
<td>3a</td>
<td>yes</td>
<td>UK, F</td>
</tr>
<tr>
<td>Cluster 10 n = 4</td>
<td>1a</td>
<td>no</td>
<td>AUS</td>
</tr>
<tr>
<td>Cluster 11 n = 4</td>
<td>1b</td>
<td>no</td>
<td>UK</td>
</tr>
<tr>
<td>Paar A,B,C n = 6</td>
<td>1a</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Paar D,E n = 4</td>
<td>1b</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Paar F n = 2</td>
<td>3a</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: NS5B phylogenetic tree of HCV subtypes 1a (left) and 4d (right).
Monophyletic clusters are shaded. Count of origin: (e) England, (n) The Netherlands, (d) Germany, (f) France, (a) Australia. Australian MSM with reported DU are marked DU.
BOOTSTRAPS WEKESEVEN.
### Introduction and spread of HCV

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Genotype</th>
<th>Nr. of sequences</th>
<th>Year of origin (CI)</th>
<th>Number (%) of divergence events since 1996</th>
<th>1996</th>
<th>1998</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>37</td>
<td>1984 (1974-1992)</td>
<td>31(86) 29(81) 20(56)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4d</td>
<td>34</td>
<td>1975 (1961-1988)</td>
<td>28(85) 26(78) 21(64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1a</td>
<td>19</td>
<td>1993 (1985-1999)</td>
<td>17(94) 16(89) 13(72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1a</td>
<td>17</td>
<td>1988 (1977-1997)</td>
<td>15(94) 14(88) 11(69)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1a</td>
<td>12</td>
<td>1996 (1989-2001)</td>
<td>11(100) 10(91) 9(82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4d</td>
<td>12</td>
<td>1995 (1989-1999)</td>
<td>10(91) 8(73) 7(64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1a</td>
<td>6</td>
<td>1998 (1990-2003)</td>
<td>5(100) 5(100) 4(80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1a</td>
<td>6</td>
<td>1983 (1970-1993)</td>
<td>1(20) 1(20) 1(20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3a</td>
<td>6</td>
<td>1984 (1974-1993)</td>
<td>0(0) 0(0) 0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1a</td>
<td>4</td>
<td>2001 (1996-2004)</td>
<td>3(100) 3(100) 3(100)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What causes this unexpected and rapid spread of HCV among HIV+ MSM?

Virus versus behaviour
# HCV geïnfecteerde MSM

<table>
<thead>
<tr>
<th>Country</th>
<th>Age</th>
<th>CD4-count</th>
<th>HAART (%)</th>
<th>IDU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK (n=107)</td>
<td>35</td>
<td>510</td>
<td>60%</td>
<td>17%</td>
</tr>
<tr>
<td>NL (n=58)</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>3%</td>
</tr>
<tr>
<td>D (n=24)</td>
<td>38</td>
<td>429</td>
<td>60%</td>
<td>4%</td>
</tr>
<tr>
<td>F (n=12)</td>
<td>40</td>
<td>604</td>
<td>75%</td>
<td>0%</td>
</tr>
<tr>
<td>AUS (n=24)</td>
<td>40</td>
<td>596</td>
<td>75%</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>518</td>
<td>62%</td>
<td>14%</td>
</tr>
</tbody>
</table>
Transmission Genotype 4

- **Red** = MSM (SC after 2000)
- **Pink** = MSM (SC before 2000)
- **Green** = Injecting drug users (IDU)
- **Orange** = Immigrants

**UNique MSM-specific HCV strain of HCV genotype 4 distinct from the strain circulating among IDU**
**Case control studie: UK (Danta et al, AIDS 2007)**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control (p)</th>
<th>Case (p)</th>
<th>Control (a)</th>
<th>Case (a)</th>
<th>P-waarde (Control)</th>
<th>P-waarde (Case)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAI (- ejaculation)</td>
<td>50%</td>
<td>90%</td>
<td>48%</td>
<td>83%</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>UAI (+ejaculation)</td>
<td>35%</td>
<td>83%</td>
<td>33%</td>
<td>58%</td>
<td>0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>Rimming</td>
<td>77%</td>
<td>98%</td>
<td>77%</td>
<td>92%</td>
<td>0.0007</td>
<td>0.03</td>
</tr>
<tr>
<td>Fisting</td>
<td>26%</td>
<td>75%</td>
<td>13%</td>
<td>58%</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Toys</td>
<td>43%</td>
<td>78%</td>
<td></td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Group sex (&gt;2pers)</td>
<td>53%</td>
<td>88%</td>
<td></td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Partydrugs</td>
<td>71%</td>
<td>97%</td>
<td></td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>
Molecular epidemiology of HCV

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Drugs use

Serosorting

Internet

Sharing use

Lower inhibitions

Mucosal damage

HIV

Rough sexual techniques

HAART-optimism

Serosorting

Groups

STI

Danta et al (2001) AIDS
The role of HIV?

**Spread:**
1) Increased susceptibility
2) Increased infectivity
3) HIV sersorting
4) Infectivity and background levels HIV vs HCV

**Clinical progression:**
1) Higher rate of chronicity (> 90%)
3) Faster progression to liver disease
4) Lees favourable treatment outcome
5) Hepatotoxicity caused by HAART
Update spread HCV among HIV+ MSM

- UK: HCV incidence among HIV+ increases 20% each year.

- HCV rare among HIV- MSM

- Increase HCV incidence **NOT** caused by intensified HCV screening

- HCV prevalence STI-clinic A’dam
  - May 2007: 15%
  - Nov 2007: 18%
  - April 2008: 21%

HCV among HIV+ MSM – current focus!

- Implement **routine HCV screening** in HIV positive MSM

- **Early diagnostics**: Early treatment and prevention of new cases

- **Targeted education and prevention** strategies to raise awareness of risks HCV among HIV+ MSM

- Monitor **HIV-negative MSM**! Are they at risk?
Thanks

Colleagues of Cluster Infectious Diseases GGD Amsterdam
Colleagues of Medical Microbiology AMC Amsterdam
HIV departments: OLVG, AMC, Slotervaart Hosp, VUMC
International Collaboration on acute HCV in HIV+ MSM
Erasmus MC and GGD Rotterdam
Roel Coutinho RIVM
Oliver Pybus University of Oxford

VHPB Organising committee