HEPATITIS B INFECTION IN GREENLAND
Epidemiology and burden of disease

PhD-thesis by
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6th Danish Pediatric Infectious Disease Symposium, Korsør 2012
Hepatitis B (HBV) Epidemiology

• 1/3 of the world’s population has positive serological markers of present or former HBV infection.

• Estimated that 350 million persons are chronic carriers
Motivation for the PhD-study

- HBV highly endemic in Greenland (HBsAg ≈ 7%, DK <1%)

- Cirrhosis and HCC less frequently observed than expected (age-adjusted male HCC incidence rate only 2 times higher than in Denmark)
  - Underreporting?
  - Benign genotypes?
  - Age at infection might be later than in other high-endemic countries?
  - Specific genetic Greenlandic constitution?

- Hepatitis B not included in the childhood vacc. program.
Studies included in the thesis

1. Hepatitis D outbreak among children in a hepatitis B hyper-endemic settlement in Greenland.

2. The effectiveness of the targeted hepatitis B vaccination programme in Greenland.

3. Incidence of hepatitis B infection, proportion of chronic carriers and HBsAg seroclearance in Greenland. A population-based longitudinal study.
   Malene L. Børresen, Mikael Andersson, Jan Wohlfahrt, Mads Melbye, Robert J. Biggar, Karin Ladefoged, Inge Panum and Anders Koch. Submitted

HBV - Natural history

1) Acute self-limiting course
   ⇒ Long-life immunity
   ⇒ HBcAb and HBsAb measured in the blood

2) Chronic course
   • The immune system is not capable of eliminating the virus
   • The virus is present in the hepatocytes and the blood
   • HBcAb and HBsAg measured in the blood

• Vaccination: ONLY HBsAb is present in the blood
Risk of chronic infection in relation to age

Risk of infection
- Perinatally ≈ 30-90%
- Later in life: dependent on viral and host factors:
  - Viral load
  - HBeAg positivity
  - Genotype?
Chronic infection – a fluctuating condition

The immune system attacks the liver
Long term consequences of chronic HBV infection

- cirrhosis

- Liver cancer (HCC, hepatocellular carcinoma)
  - Life risk between 1-30% (genotype, mutations, age at infection, male, genetic susceptibility)

- App. 15-25% of HBV chronic infected will die from liver related diseases
Study III + IV - METHODS

8,879 persons followed for 151,000 person-years (PY)

Results from the Greenlandic Hepatitis B Database (1992-2009)
Results from the Incidence Notification Database (1987-1991)

Follow-up 2008/9

6,267 persons
2,716 persons

1987 1998 2009
Hepatitis B relateret sygelighed

<table>
<thead>
<tr>
<th>Hospitalizations</th>
<th>All 8,879</th>
<th>Chronic carriers 650</th>
<th>HBV-Negative 5,160</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>(Adjusted IRR)</td>
<td>N</td>
</tr>
<tr>
<td>All liver-related Diseases</td>
<td>117</td>
<td>31</td>
<td>5.73 (3.52, 9.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCC</td>
<td>15</td>
<td>5</td>
<td>8.70 (2.06, 36.7)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>17</td>
<td>4</td>
<td>4.52 (1.23, 16.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronisk hepatitis</td>
<td>47</td>
<td>18</td>
<td>11.4 (5.40, 23.9)</td>
</tr>
<tr>
<td>Alc. liver-disease</td>
<td>24</td>
<td>1</td>
<td>0.51 (0.07, 3.99)</td>
</tr>
<tr>
<td>Alcoholisme</td>
<td>618</td>
<td>52</td>
<td>1.12 (0.83, 1.51)</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>242</td>
<td>28</td>
<td>1.64 (1.08, 2.50)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>137</td>
<td>16</td>
<td>2.27 (1.28, 4.04)</td>
</tr>
<tr>
<td>Female gen. cancer</td>
<td>86</td>
<td>11</td>
<td>1.87 (0.89, 3.92)</td>
</tr>
</tbody>
</table>

IRR, Incidence rate ratios
### Age Standardized Incidence Rates (ASR)

<table>
<thead>
<tr>
<th>Location</th>
<th>HCC</th>
<th>Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Greenland</strong></td>
<td>38.5/100,000</td>
<td>24/100,000</td>
</tr>
<tr>
<td><strong>Sweden, Alaska, China, Taiwan</strong></td>
<td>65-225/100,000</td>
<td>100-600/100,000</td>
</tr>
</tbody>
</table>

**Hepatocellular Carcinoma and Other Liver Disease Among Greenlanders Chronically Infected with Hepatitis B Virus: A Population-Based Study.**

Study I
Hepatitis B and D outbreak in the settlement Itilleq

ML Børresen et al.
Study I

Results 2006-2007

90% (122/135 persons) tested

Overall sero-prevalence
- 27% chronic infected
- 56% immune
- 17% never exposed

94% (52/54) of children tested
- 29% chronic infected
- 35% immune
- 37% never exposed
Severity markers for HBsAg-positive, 2006-2007

<table>
<thead>
<tr>
<th></th>
<th>Children (n=15) (%)</th>
<th>Adults (n=16) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT &gt; 45 I/U</td>
<td>73</td>
<td>38</td>
</tr>
<tr>
<td>Viral load &gt; 1 mio. IU/mL</td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>HBeAg positive</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis D (HDV) positive</td>
<td>40</td>
<td>63</td>
</tr>
<tr>
<td>HDV- seroconversion</td>
<td>33</td>
<td>0</td>
</tr>
</tbody>
</table>

Regression model: Hepatitis D the strongest predictor for elevated ALT (liver damage) 
In 2009, additional 2 children HDV seroconverted
HBV in Itilleq – Conclusions

- High prevalence of chronic HBV infection, especially among children (genotype D)
- Elevated liver enzymes in chronic infected (HBeAg-positive) children
- Super-infection with Hepatitis D most likely, (clade I)
- Ongoing HDV outbreak in Itilleq
By September, 2010 the HBV vaccine was included in the childhood vaccination program in Greenland.
Evaluation of HBV Vaccination in Greenland before 2010

- Included: 207 children (83% of all at-risk children from 1992-2009) born to HBsAg positive mothers
  - Information on vaccination coverage
- Included: 140 (66%) of children born to HBsAg positive mothers
  - Prevalence of break-through infections among vaccinated children of HBsAg positive mothers
  - Levels of protective antibodies among HBV-negative children.

Conclusions

- 20% of at-risk children received no vaccination postnatally
- Only 30% received full vaccination program
- 6% had breakthrough infections, most occurring in children with at least three vaccinations, and half of these infections resulted in chronic infection.
- 59% of HBcAb-negative children with 3+ vaccinations had HBsAb < 10 IU/l
- 73% of all included children had HBsAb < 10 IU/L
Reasons for low HBsAb level in vaccinated children?

- Vaccine quality – cold chain?
- Escape mutants: HBV strains in Greenland may contain mutations in the ‘a’ determinant of the gene encoding for HBsAg?
  => infection despite vaccination
- High frequency of poor responders to HBV vaccine in Greenland?
  - Genetic constitution (host/virus)
  - Environmental factors
Vaccine quality

- Statens Serum Institut’s Quality-Control Department investigated the storage facilities and distribution pattern at different airports and storages in Greenland in 2010 and found no reasons to question the chain
Escape mutants in HBV strains
HBV strain sequencing

- From HBsAg positive children, hereof 3 siblings and a sister who was HBsAg negative and HDV positive
- We found no mutations associated with immune escape but specific changes with stop mutation in the pre-s and post-s region

Done by Carla Osiowy and Kaarina Solar, Manitoba, Canada
HBV vaccination breakthrough infections

- **GL8 Mother of Family 4**
  - Born 1998
  - FAMILY 1 (GL9)
  - HBsAg +
  - HBV DNA: 9.3 x 10^7 IU/ml
  - ALT: 56
  - HDV: neg

- **5192 ♀ Born 1993**
  - FAMILY 4
  - HBsAg+, anti-HBe+
  - ALT: 358
  - HDV: pos

- **5193 ♀ Born 1994**
  - FAMILY 4 (GL4)
  - HBsAg+, HBeAg+
  - HBV DNA: 5.1 x 10^7 IU/ml
  - ALT: 357
  - HDV: pos

- **5185 ♀ Born 2003**
  - FAMILY 4
  - HBsAg+, HBeAg+
  - ALT: 321
  - HDV: pos

- **1527 ♀ Born 1998**
  - FAMILY 1 (GL9)
  - HBsAg +
  - HBV DNA:
    - 9.3 x 10^7 IU/ml
  - ALT: 56
  - HDV: neg
  - Label 3:133

- **5192 ♀ Born 1993**
  - FAMILY 4
  - HBsAg+, anti-HBe+
  - ALT: 358
  - HDV: pos

- **5193 ♀ Born 1994**
  - FAMILY 4 (GL4)
  - HBsAg+, HBeAg+
  - ALT: 357
  - HDV: pos

- **1529 ♂ Born 1996**
  - FAMILY 3 (GL6)
  - ALT: 54
  - HDV: neg

- **1585 ♀ Born 1999**
  - FAMILY 1 (GL15)
  - HBsAg +
  - ALT: 56
  - HDV: neg

- **56 clones**

- **79 clones**

- **93 clones**

- **88 clones**

- **3 siblings having vaccine breakthrough**

- **not vaccinated**

- **not vaccinated**

- **not vaccinated**

- **88 clones**

- **79 clones**

- **93 clones**

- **56 clones**

- **3 siblings having vaccine breakthrough**

- **not vaccinated**

- **not vaccinated**

- **not vaccinated**

**MENITSG**

**FLGPLLVLQAGFFLLTRIL**

**TIPQSLDSWWTSLNFLGGT**

**TTVCLGQNSQSPTSNSHPTSCPPTC**

***PGYRWMCLRRFIIFLFI**

**LLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGCPRTCJTPAQGTSMY**

**PSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVVFVFQWVFVGLSPTVWLSVIMMMWAYWGPSLYSILSPFLP**

**LL**

**PIFFCLWVYI.**

(F8L, G44E, Y134F)
Occult infection?

HBcAb-positive, HBsAg-negative relatives to chronic infected

- HBV-DNA PCR on 63 HBcAb positive, HBsAg negative "immune" individuals from Itilleq and Kangaatsiaq

- 6 "positives"
  - 2 persons
    - 0.4 and 0.5 $10^3$ IU/ml
  - 4 persons weak positive

<table>
<thead>
<tr>
<th></th>
<th>Siblings parents</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA positive</td>
<td>2*</td>
<td>4*</td>
</tr>
<tr>
<td>HBV DNA negative</td>
<td>13</td>
<td>44</td>
</tr>
</tbody>
</table>
Poor responders?
Hepatitis B and the immune system

- Balanced Th1- og Th2-response plays a role for the antibody response when vaccinated (Rehermann, 2003)

- 5-10% of newborns are non-responders (Zuckerman, 2006)
  - lower TH1 og TH2 response
  - Defect in the HBsAg specific T-cells (Velu, 2008)
  - Immunological tolerance
  - External factors
Hepatitis B, Greenlanders and the immune system

External factors that can modulate the immune system?

• **Persistereende Organic Pollutants (POP’s)**
  - Lower vaccine response in relation to cumulative PCB exposure
    (Faeroe Islands, Heilmann, 2006)
  - Immune modulators
    (Ebketar, 2004)
  - Increased risk of infections in children is related to POP-exposure in mothers milk
    (Canada, Daillaire, 2006; Holland, Weisglas-Kuperus, 2004)

• **Organic Perfluorinated Compounds (PFC)**
  Grandjean et al., Faeroe Islands:
  - Correlation between levels of (PFC) and level of antibodies against diphtheria and tetanus at the age of 5 years
    (Granjean, JAMA, 2012)
Hepatitis B, Greenlanders and contaminants

- Level of PCF and POP’s are high in the Greenlandic population due to the intake of fish and whale, especially in the settlements and more rural areas (Butt, 2010)

- Smoke induces lower metabolisation of POP’s – in 1999 70% of the Greenlandic population smoked
  - Mother smoking during pregnancy => the newborn has decreased immunological response
Vaccine induced HBsAb level and PFC’s

Aim

- Relation between vaccine induced HBsAb, tetanus and diphtheria antibodies
- Relation between PFC’s in the blood and the HBV vaccination response among HBV vaccinated children

Material

- Cohort of 69 HBV vaccinated children (3 or 4 vaccinations as infants)
Tetanus antibodies by age

BOOSTER DOSE?

Tetanus Ab

Age

Tetanus Ab
Diphteria antibodies by age

BOOSTER DOSE?

Diphtheria Ab

Diphtheria Ab

Age
Tetanus by Hepatitis B antibodies
Diphtheria by Hepatitis B antibodies

Difteria

HBsAb

HBsAb

0 0,5 1 1,5 2 2,5 3 3,5

0 5 10 15 20 25 30 35 40 45
Conclusions so far

• 59% of HBcAb-negative children with 3+ vaccinations had HBsAb < 10 IU/l

• NON – RESPONDERS?

• Low tetanus and Diphtheria levels in relation to low Hepatitis B antibodies and age.
  • Were they boosted?

• Next step antibody levels and contaminants
PhD Headlines

- Prevalence of HBsAg in Greenland app. 7% - with large regional differences

- Hepatitis D outbreaks occur in Greenland – the infected younger move around

- The focused vaccination program did not work sufficiently
  - Non-responders..

Cirrhosis and HCC
- Chronic carriers have 4-8 times higher risk than HBV-negative individuals

But
- The Standardized Incidence Rate low as compared with population-based studies from low, intermediate and high-endemic countries
Public health implications

- Chronic carriers in Itilleq are now followed on a regular basis.
- Chronic carriers in Greenland are planned to be followed.
- Sep. 2010, HBV vaccination was included in the Childhood Vaccination Program in Greenland (birth, 3 month, 5 month and 12 month)
- HBV vaccination in infancy will protect against infection in adulthood
- Prevention of HBV infection will also prevent spread of HDV
Thank you

Thank you to all participants and collaborators

- Anders Koch, Jan Wohlfahrt, Karin Ladefoged og Mads Melbye
- Mikael Andersen, Statistiker
- Annemette Kristensen, Helle Jørgensen, Jytte Larsen og Anders Nielsen, SSI
- Henning Sloth Pedersen, lægeklinikken, DIH og Århus Universitet
- Ove Rosing Olsen, Chefdistriktslæge Sisimiut
- Mathias Hertz (Medicinstuderende, Kbh.) og Ajannuaq Enoksen (tolk, Sisimiut sygehus)
- Thomas Rendal, Sygeplejerske, DIH
- Inge-Lise Kleist, Chef-bioanalytiker, DIH
- Flemming Stentz, Landslæge
- Annelise G Nielsen, Allan Hansen, Ulla Sørensen, Lene Waltoft og andet personale på Virologisk Afdeling, SSI
- Carla Osiowy, Mannitoa, Canada
- Henrik Krarup, Ålborg University hospital
- Brian McMahon, Anchorage, Alaska

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Tak for opmærksomheden