STATENS SERUM INSTITUT

HEPATITIS B INFECTION IN GREENLAND
Epidemiology and burden of disease

PhD-thesis by

Malene Landbo Børresen

Dep. of Epidemiological Reseach

Statens Serum Institut

Supervisors:

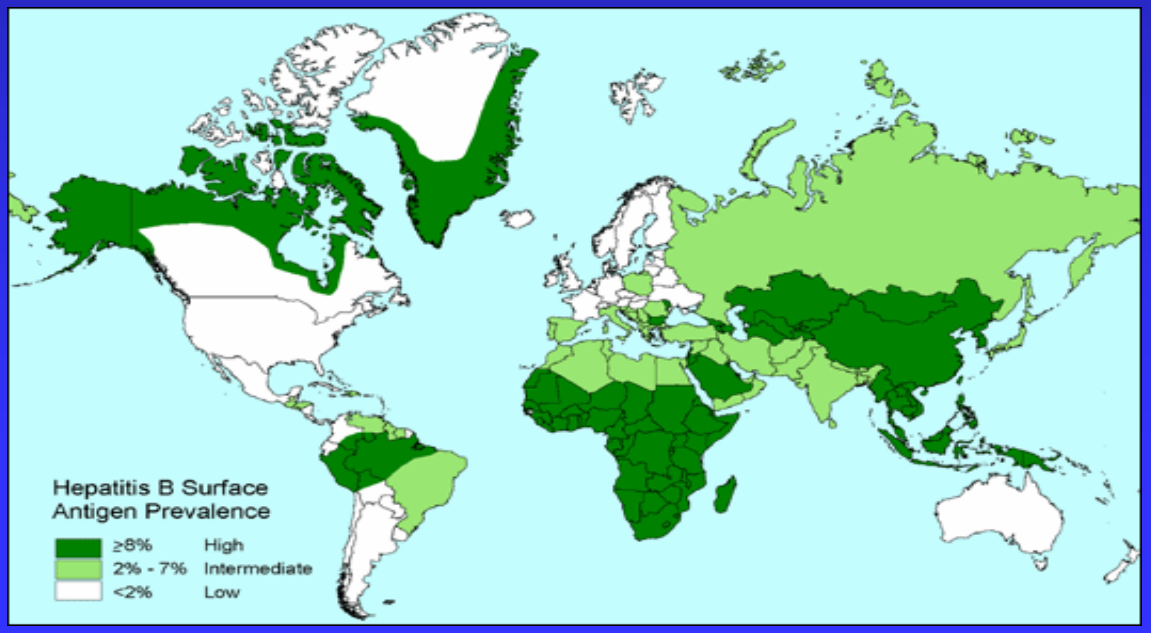
SSI: Anders Koch, Jan Wohlfahrt og Mads Melbye

DIH: Karin Ladefoged

6.th 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

STATENS SERUM INSTITUT

- 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

STATENS SERUM INSTITUT

Children and vaccination

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

   Malene L Børresen, Ove Rosing Olsen, Karin Ladefoged, Brian J McMahon, Thomas Hjuler, I Panum, Josephine Simonetti, Carla Jones, Henrik Krarup, and Anders Koch. J *Viral Hepat. 2010 Mar;17(3):162-70.*

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

   Malene L. Børresen, Anders Koch, Robert J Biggar, Karin Ladefoged, Mads Melbye, Jan Wohlfahrt, and Tyra Grove Krause. *Am J Public Health. 2011 Sep 22.*

Incidence and cancer

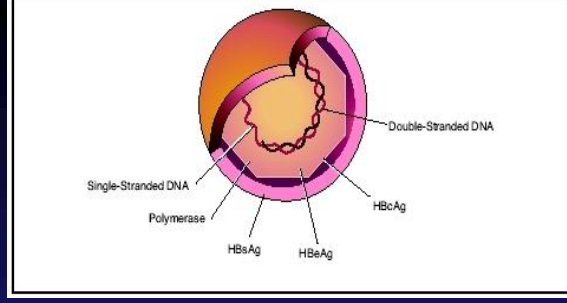
3. **Incidence of hepatitis B infection, proportion of chronic carriers and HBsAg seroclearance in Greenland. A population-based longitudinal study.**

   Malene L. Børresen, Mikael Andersson, Jan Wohlfahrt, Mads Melbye, Robert J. Biggar, Karin Ladefoged, Inge Panum and Anders Koch. *Submitted*

4. **Incidence of hepatocellular carcinoma and other liver disease among Greenlanders chronically infected with hepatitis B virus. A population-based study.**

   Malene L. Børresen, Anders Koch, Robert J. Biggar, Mikael Andersson, Jan Wohlfahrt, Karin Ladefoged and Mads Melbye. *J Nat. Cancer Inst. 2011 Oct 21.*

HBV - Natural history



Double-stranded DNA virus of the Hepadnaviridae family

1) Acute self-limiting course

⇒ Long-life immunity

⇒ HBcAb and HBsAb measured in the blood

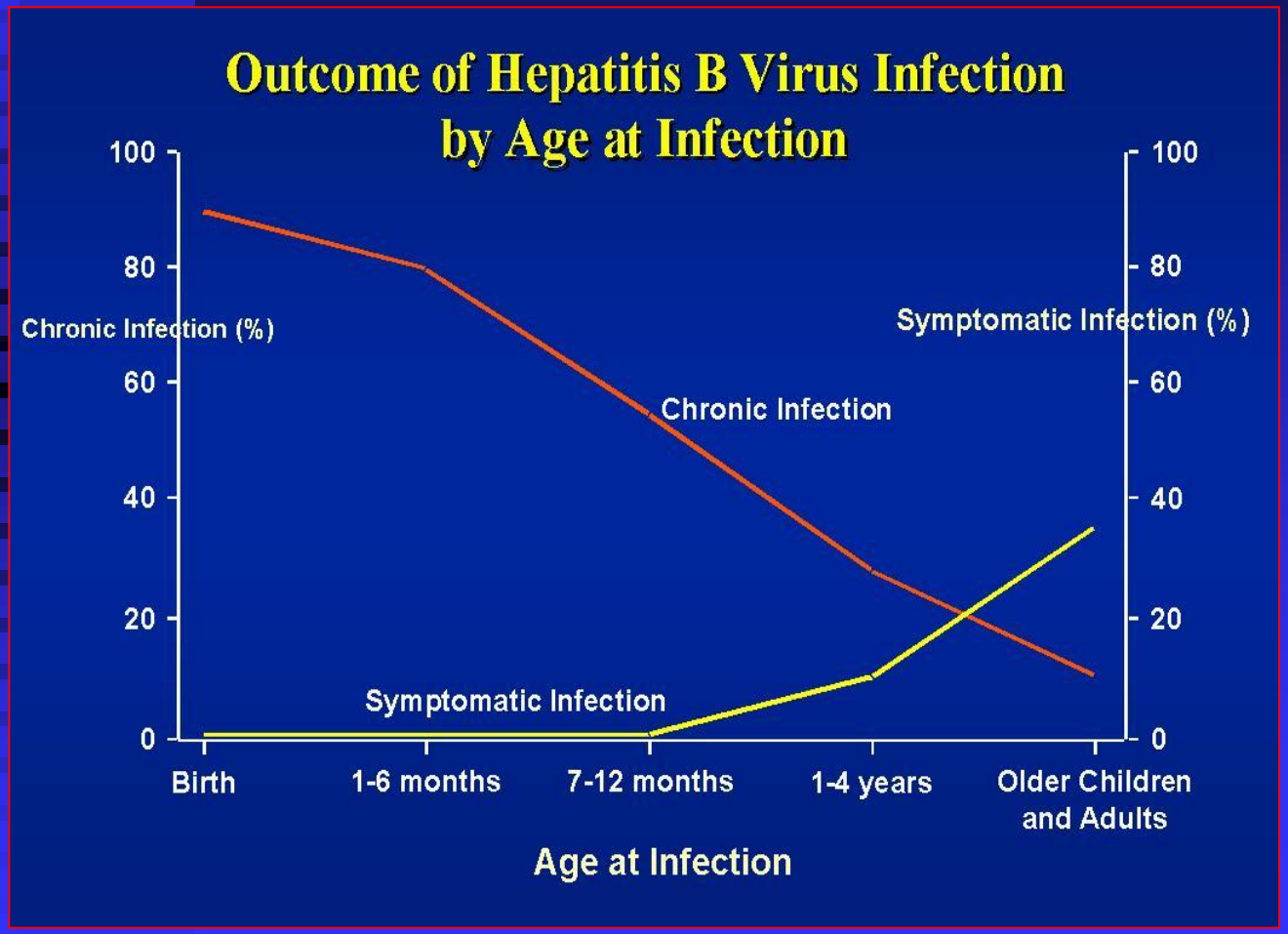
2) Chronic course

- 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

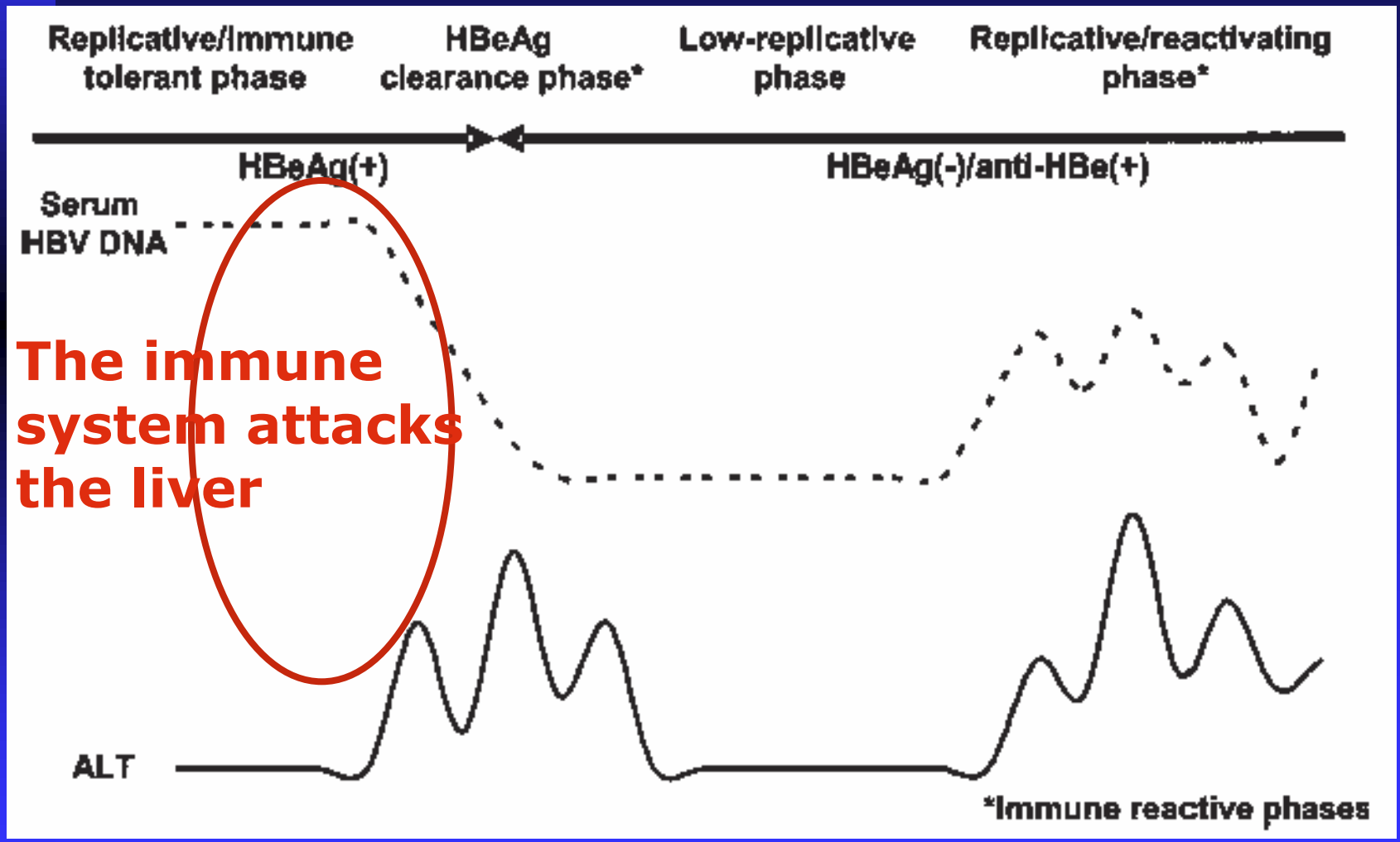
STATENS SERUM INSTITUT



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

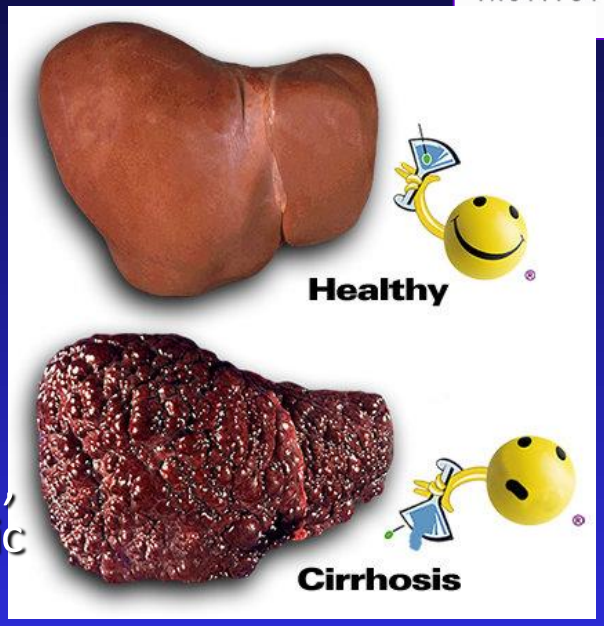


Hadziyannis et al Semin.Liver Dis.2006.

Long term consequences of chronic HBV infection

STATENS SERUM INSTITUT

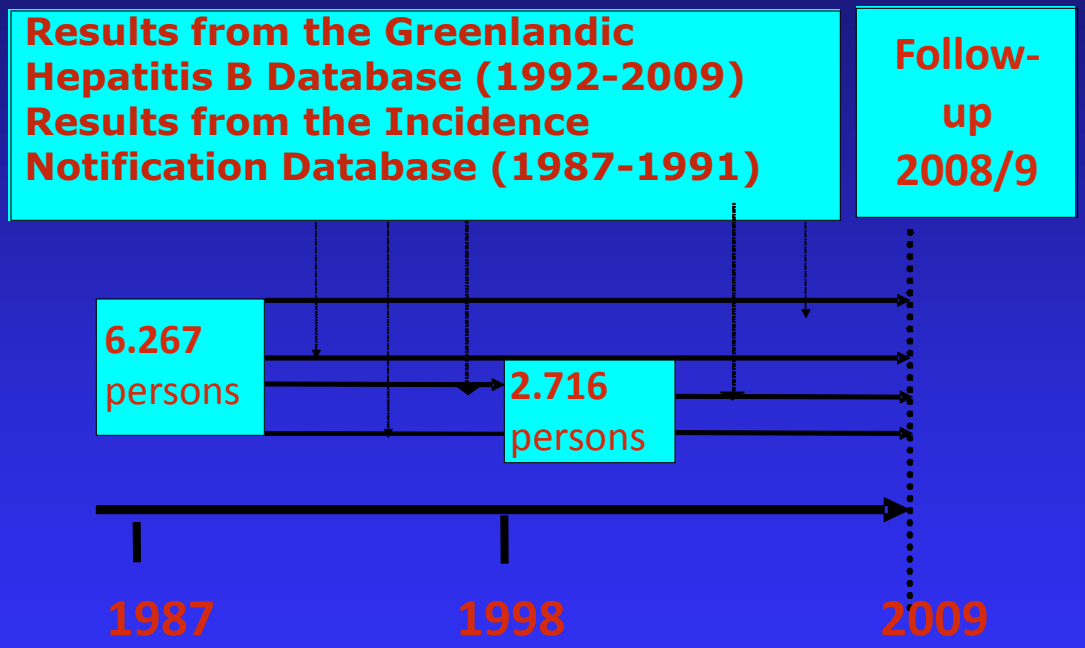
- cirrhosis
- Liver cancer (HCC, hepatocellular carcinoma)
  - Life risk between 1-30% (genotype, , mutations ,age at infection, male, genetic suseptability)
- App. 15-25% of HBV chronic infected will die from liver related diseases



Study III +IV - METHODS

8.879 persons followed for 151.000 person-years (PY)

STATENS SERUM INSTITUT



Hepatitis B relateret sygelighed

STATENS SERUM INSTITUT

Hospitalizations	All 8,879	N	Chronic carriers 650 (Adjusted IRR) Rate Ratio	N	HBV-Negative 5,160 Reference
All liver-related Diseases	117	31	5.73 (3.52, 9.34)	41	1
HCC	15	5	8.70 (2.06, 36.7)	3	1
Cirrhosis	17	4	4.52 (1.23, 16.7)	7	1
Chronisk hepatitis	47	18	11.4 (5.40,23.9)	12	1
Alc. liver-disease	24	1	0.51 (0.07, 3.99)	15	1
Alcoholisme	618	52	1.12 (0.83, 1.51)	321	1
Tuberculosis	242	28	1.64 (1.08, 2.50)	103	1
Lung cancer	137	16	2.27 (1.28, 4.04)	54	1
Female gen. cancer	86	11	1.87 (0.89, 3.92)	38	1

IRR, Incidence rate ratios

Age Standardized Incidence Rates (ASR)

STATENS SERUM INSTITUT

Greenland

- HCC
  38.5 /100.000
- Cirrhosis
  24/100.000

Sweden, Alaska, China, Taiwan

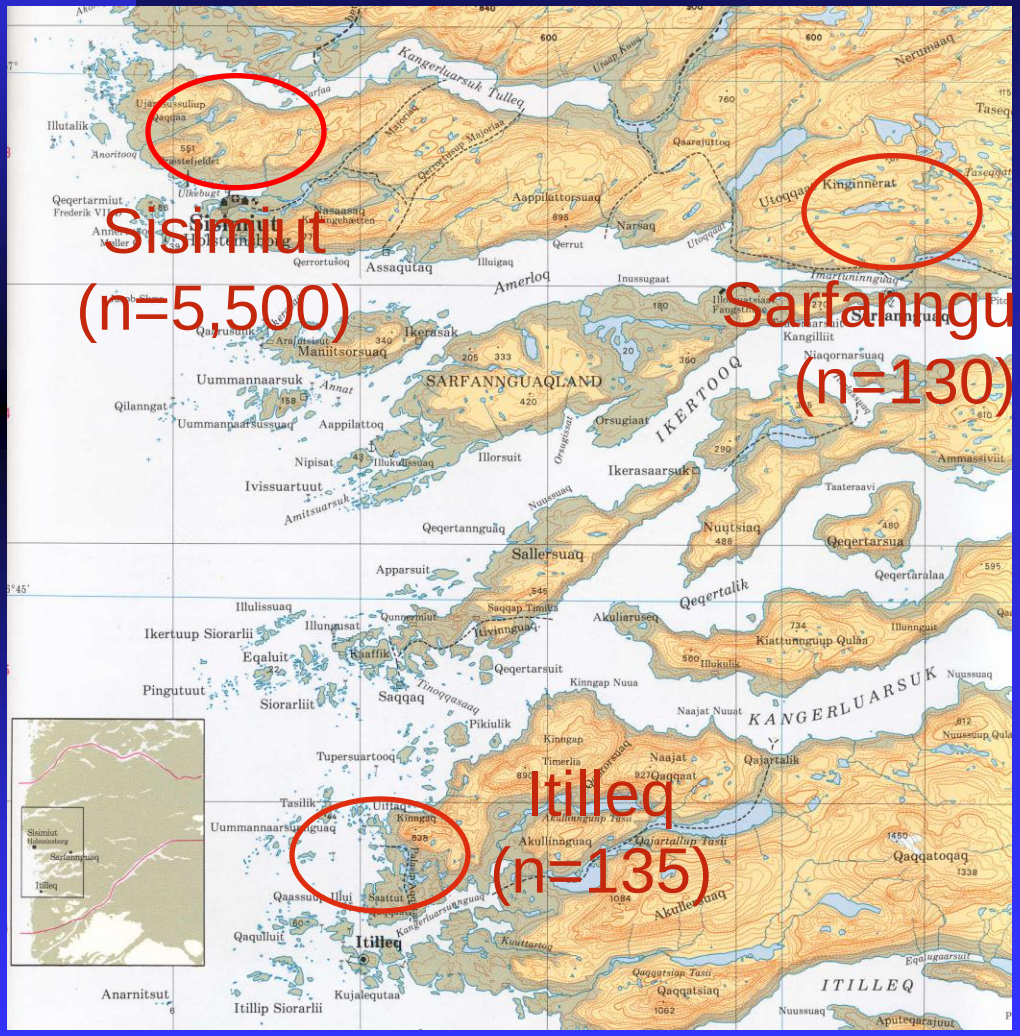
- HCC
  65-225/100.000
- Cirrhosis
  100-600/100.000

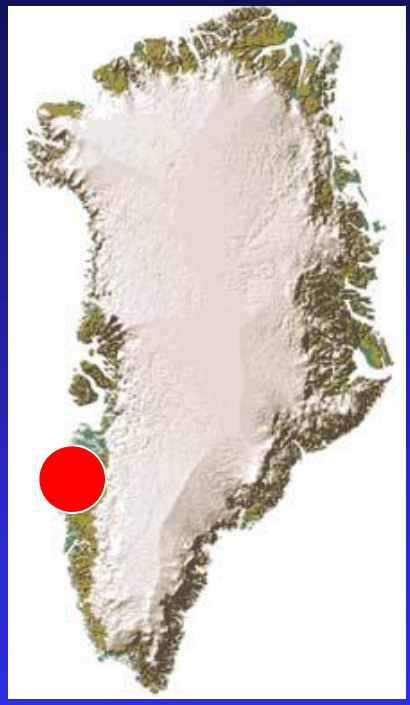
Hepatocellular Carcinoma and Other Liver Disease Among Greenlanders Chronically Infected with Hepatitis B Virus: A Population-Based Study.
Børresen ML el al. J Natl Cancer Inst. 2011 Oct 21.

Study I

Hepatitis B and D outbreak in the settlement Itilleq

STATENS SERUM INSTITUT





ML Børresen et al. Hepatitis D outbreak among children in a hepatitis B hyper-endemic settlement in Greenland. Journal of Viral Hepatitis, 2009, Vol. 17, Issue 3, Pages 162 - 170

Study I

Results 2006-2007

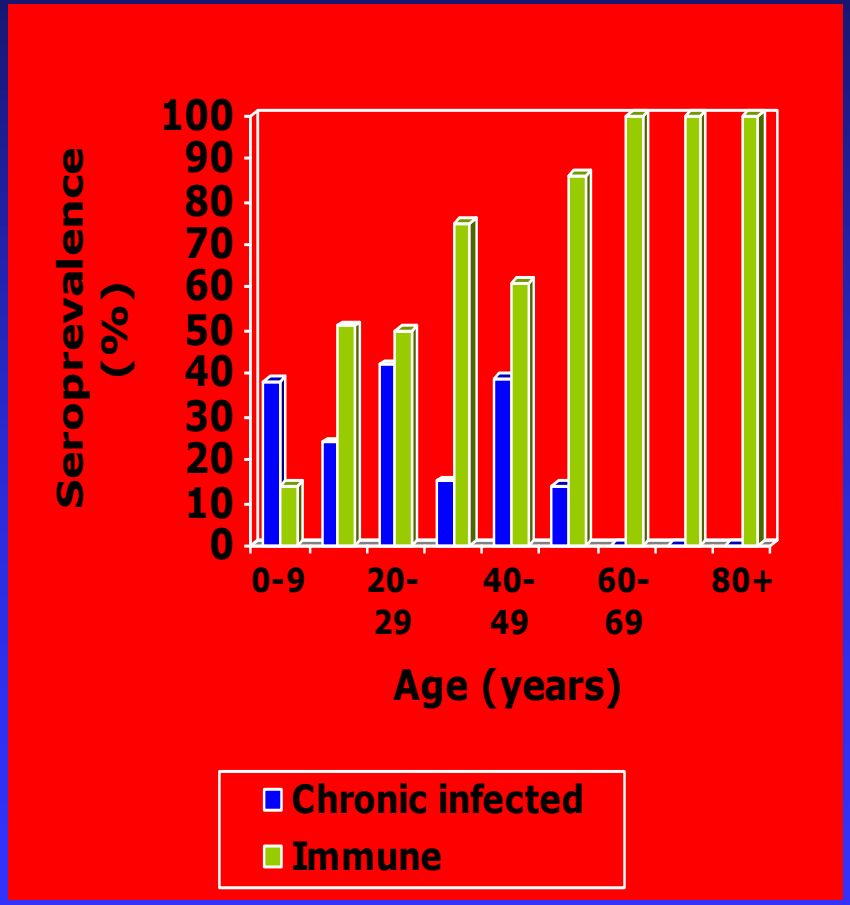
90% (122/135 persons) tested

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

STATENS SERUM INSTITUT

	Children (n=15) (%)	Adults (n=16) (%)
ALT > 45 I/U	**73**	**38**
Viral load> 1 mio. IU/mL	47	6
HBeAg positive	53	0
Hepatitis D (HDV) positive	40	63
HDV-seroconversion	**33**	**0**

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

HBV in Itilleq – Conclusions

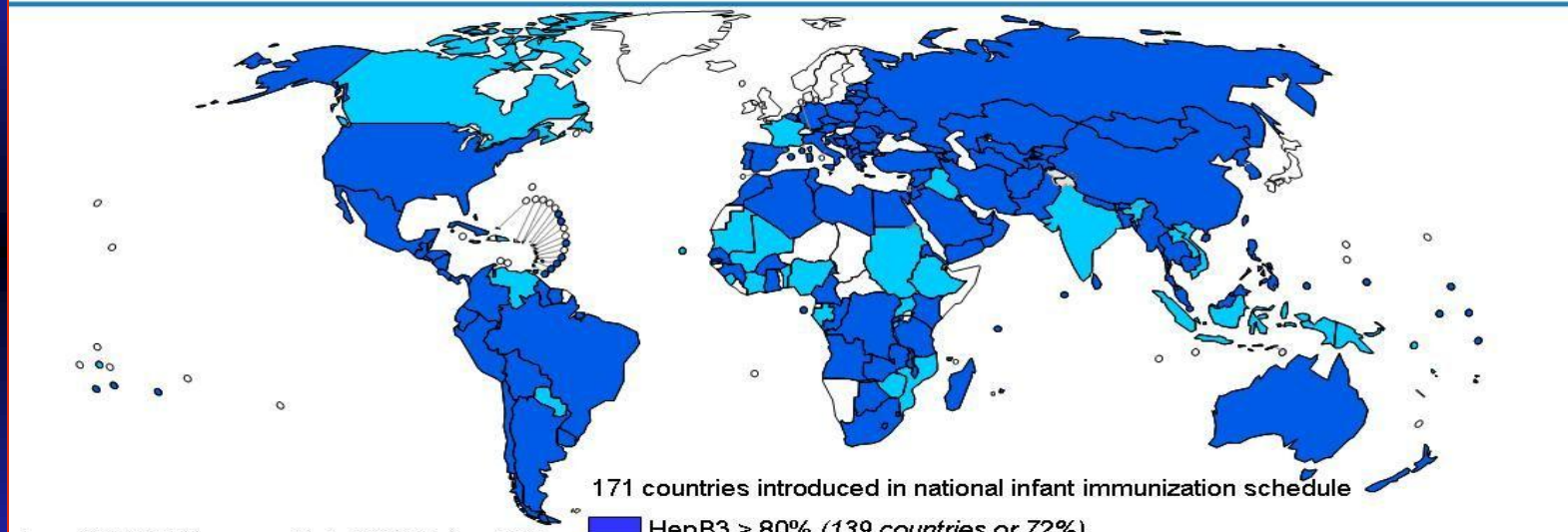
STATENS SERUM INSTITUT

- 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

Hepatitis B Vaccine Coverage

STATENS SERUM INSTITUT

Countries having introduced HepB vaccine and infant HepB3 coverage, 2007



Source: WHO/UNICEF coverage estimates 1980-2007, August 2008

Date of slide: 4 September 2008

World Health Organization

By September, 2010 the HBV vaccine was included in the childhood vaccination program in Greenland

Evaluation of HBV Vaccination in Greenland before 2010

STATENS SERUM INSTITUT

- 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.

Børresen ML, Koch A, Biggar RJ, Ladefoged K, Melbye M, Wohlfahrt J, Krause TG.
Am J Public Health. 2012 Feb;102(2):277-84.

Conclusions

Vaccination

- 20% of at-risk childen 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?

STATENS SERUM INSTITUT

- 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)
  - Enviromental factors

Vaccine quality

STATENS SERUM INSTITUT

- 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

STATENS
SERUM
INSTITUT

Escape mutants in HBV strains

HBV strain sequencing

STATENS SERUM INSTITUT

- 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, Mannitoba, Canada

HBV vaccination breakthrough infections

STATENS SERUM INSTITUT

GL8 Mother of Family 4

not vaccinated

3 siblings having vaccine breakthrough

not vaccinated

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

88 clones

Label 3:133 ♀
Born 1999
FAMILY 1 (GL15)
- HBsAg +
- HBV DNA: 2.8 x 10^7 IU/ml
- HDV:pos in '09

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

*Stop mutant present at pos 216

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

79 clones

*Stop mutant present at low level (3%)

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

93 clones

*Stop mutant present at high level (70%) at pos. 69
Mutant also found in 2623, from Kangaatsiaq

1529 ♂
Born 1996
FAMILY 3 (GL6)
- HBsAg +
- HBV DNA: 5.0 x 10^7 IU/ml
- ALT: 54
- HDV:neg

56 clones

Label 3:10 ♀
Born 1990
FAMILY 3 (GL3)
- HBsAg +
- HBV DNA: 1.5 x 10^7 IU/ml
- ALT: 253
- HDV:pos

MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGG
TTVCLGQNSQSPTSNHSPTSCPPTC*PGYRWMCLRRFIIFLFIL
LLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCTTPAQGT
SMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSW
LSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYSILSPFLP
LL*PIFFCLWVYI. (F8L, G44E, Y134F)

Occult infection? HBcAb-positive, HBsAg-negative relatives to chronic infected

STATENS SERUM INSTITUT

- 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

	Siblings parents	Others
HBV DNA positive	2*	4*
HBV DNA negative	13	44

STATENS
SERUM
INSTITUT

Poor responders?

Hepatitis B and the immune system

- Balanced Th1- og Th2-response plays a role for 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

STATENS SERUM INSTITUT

External factors that can modulate the immune system?

- Persisterende 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

STATENS SERUM INSTITUT

- 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

STATENS SERUM INSTITUT

Aim

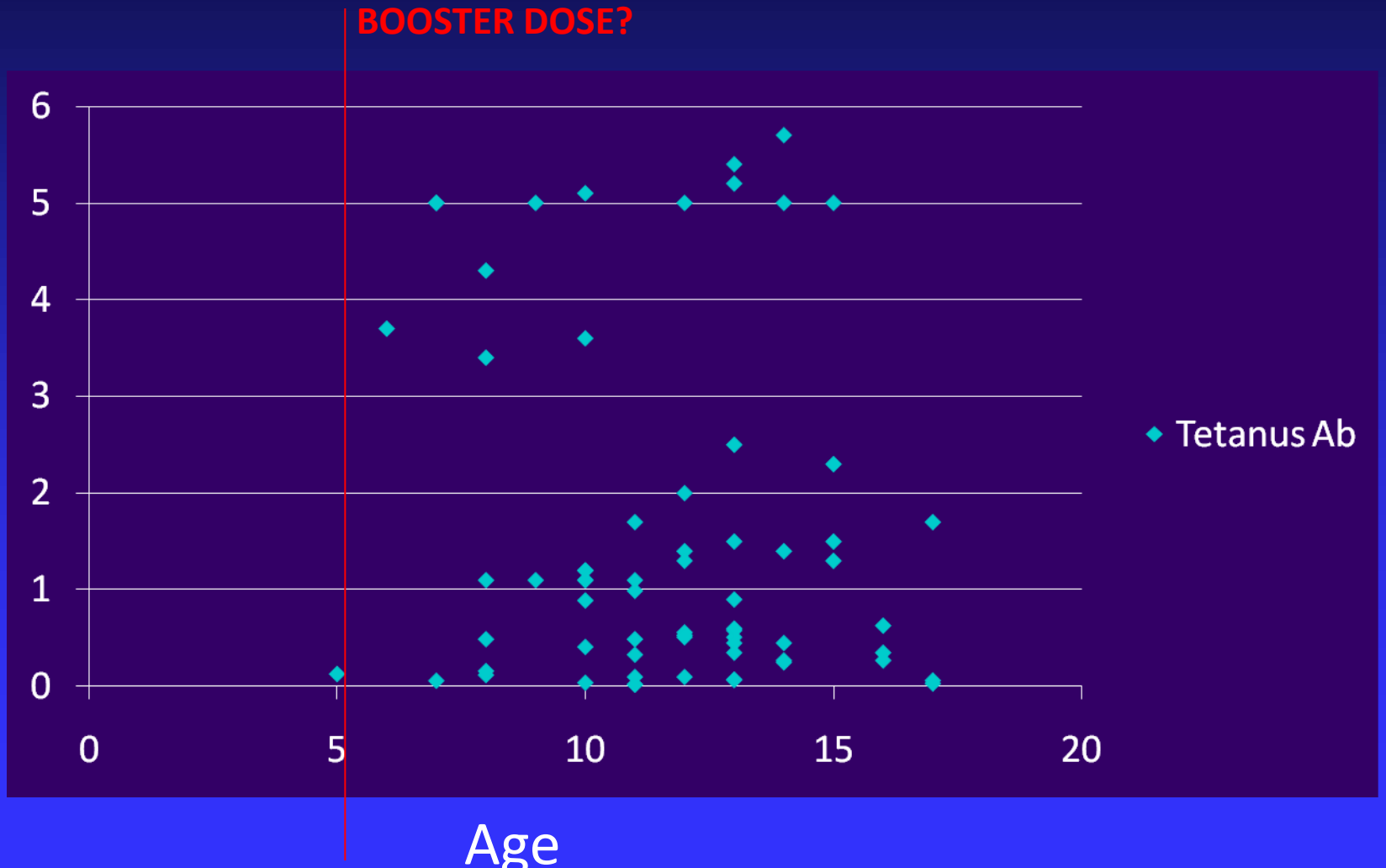
- Relation between vaccine induced HBsAb, tetanus and diphtheria antibodies
- Relation between PFC's in the blood and the HBV vaccination response among HBV vaccinated children

Material

- Cohort of 69 HBV vaccinated children (3 or 4 vaccinations as infants)

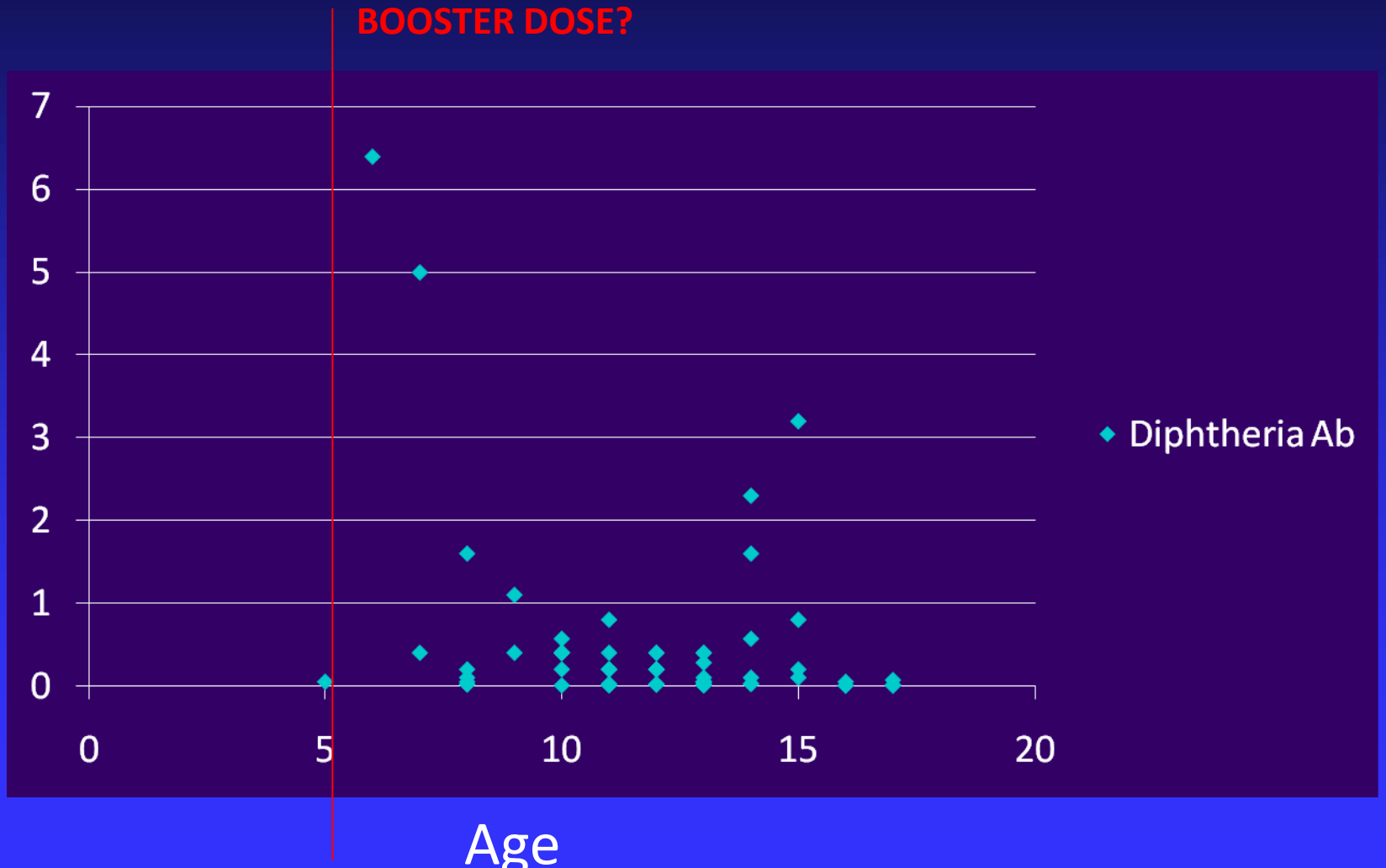
Tetanus antibodies by age

STATENS SERUM INSTITUT



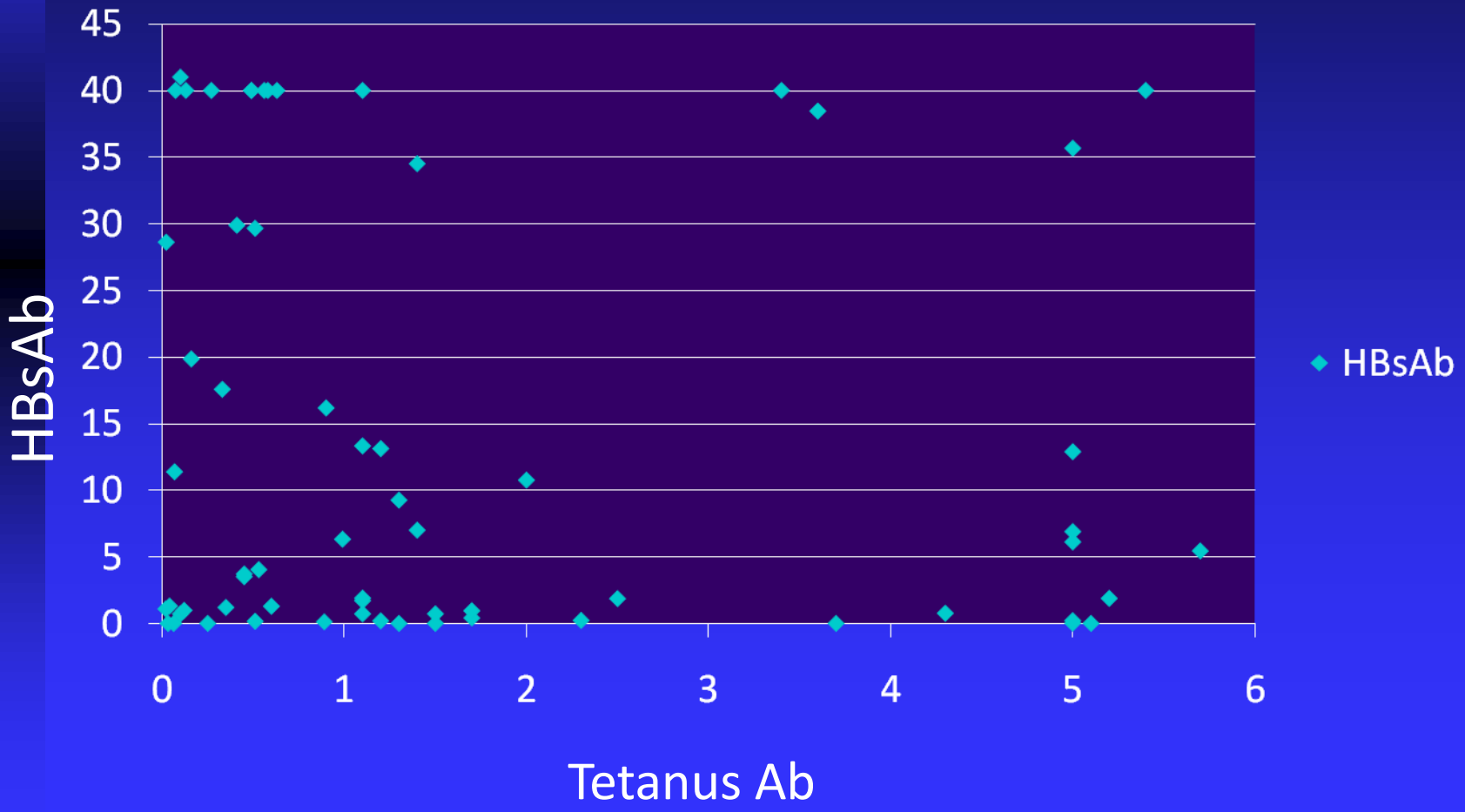
Diphteria antibodies by age

STATENS SERUM INSTITUT



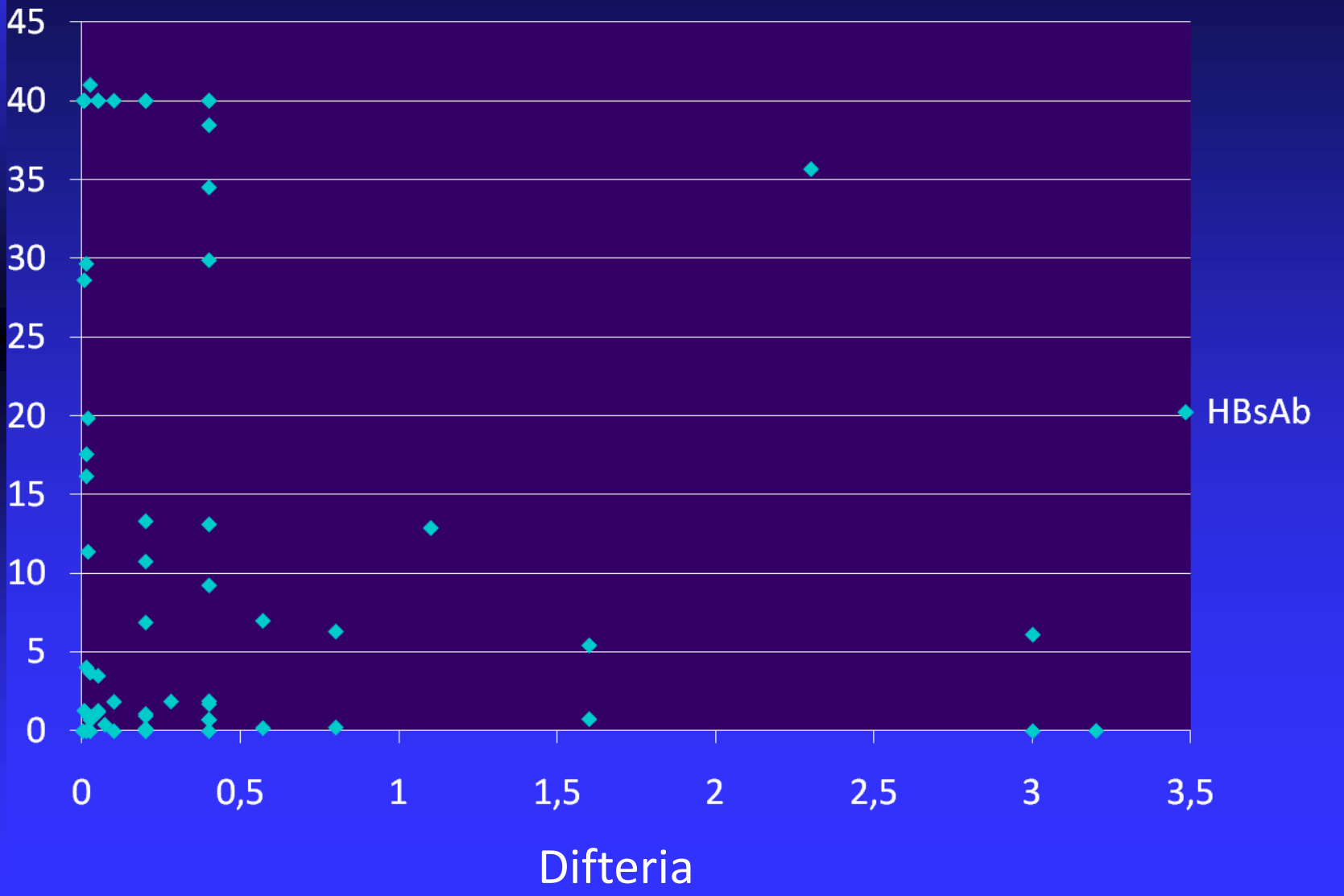
Tetanus by Hepatitis B antibodies

STATENS SERUM INSTITUT



Diphtheria by Hepatitis B antibodies

STATENS SERUM INSTITUT



Conclusions so far

STATENS SERUM INSTITUT

- 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 i 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

STATENS SERUM INSTITUT

- 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

STATENS SERUM INSTITUT

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**

Malene L Børresen: mlb@ssi.dk


Tak for opmærksomheden