Perinatal HBV viremia in newborns of HBsAg(+) mothers is a transient phenomenon that does not necessarily imply HBV infection transmission

Vana Papaevangelou (Greece)

National and Kapodistrian University of Athens

Hepatitis B virus MTCT in the era of passive-active immunoprophylaxis

- **Failure of immunoprophylaxis**
  - 5%–10% of infants of HBsAg+ mothers
  - Recent meta-analysis (>7500 Chinese babies):

  4.87% in infants born to HBsAg(+) and 9.66% in infants born to HBeAg(+) mothers respectively
- **Why?**
  - HBV intrauterine infection
    - have an established infection at birth
  - Perinatal transmission post poor adherence to administration of immunoprophylaxis and/or timely administration of HBV vaccination

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Lin X et al PIDJ 2014

Hepatitis B virus MTCT in the era of passive-active immunoprophylaxis

- Transplacental (in - utero) transmission has been associated with:
  - **HBeAg (+) mother,**
  - **High maternal HBV DNA** (>10^6 copies/mL),
  - High maternal HBsAg titer,
  - HBV genotype B versus C,
  - male fetus, amniocentesis, pregnancy complications or prolonged labor,
  - antigenemia in siblings

Chang MH SFNM 2007
ZX Li et al. Emerging Microbes and Infections 2015

Diagnosis of HBV infection in infants

- **HBsAg (+) infants for > 6months**

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Zhu Q, et al. Chin Med J (Engl) 2003

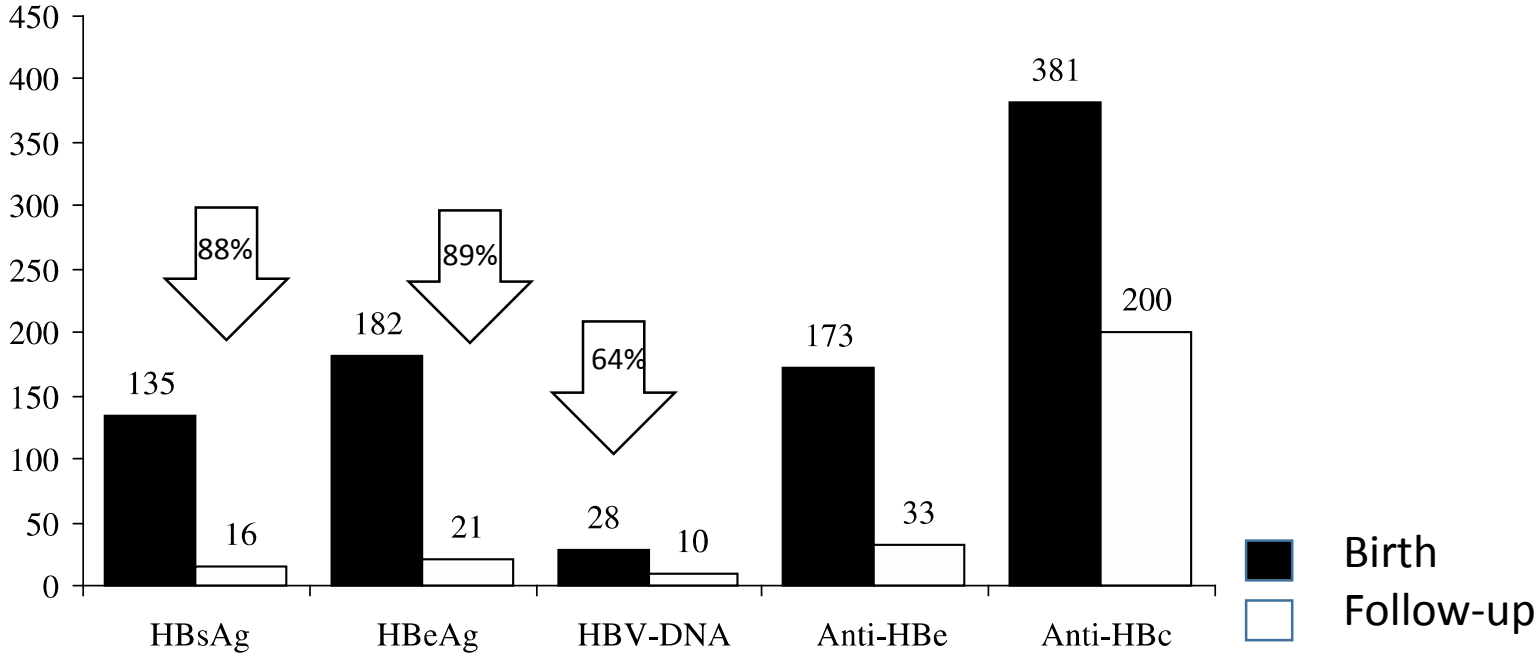
Diagnosis of HBV infection in infants

- **HBsAg (+) infants for > 6months**
- HBsAg/HBeAg/HBVDNA positivity in the cord blood (? contamination)
- HBV seromarkers and HBV DNA in venous blood persist in older infants?

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Zhu Q, et al. Chin Med J (Engl) 2003

Positive HBV markers at birth do not necessarily indicate in-utero transmission

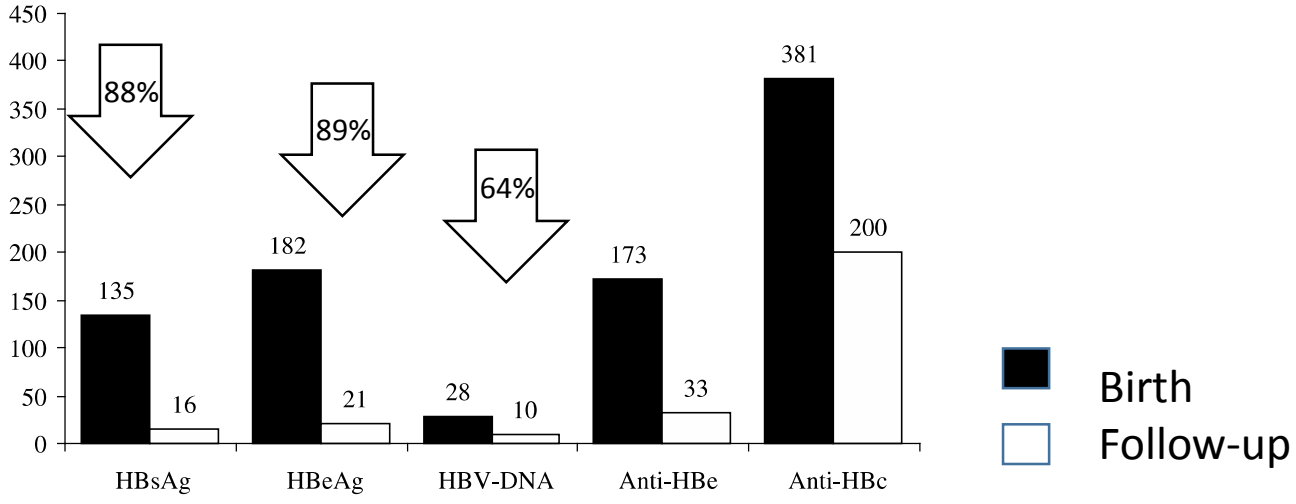
- 385 neonates born to HBsAg (+) mothers followed for 8-12 months.
- Femoral vein (FV) and umbilical cord (UC) blood samples obtained before immunoprophylaxis.



Zhang et al. BMC ID 2016

Positive HBV markers at birth do not necessarily indicate in-utero transmission

- 385 neonates born to HbsAg (+) mothers followed for 8-12 months.
- Femoral vein (FV) and umbilical cord (UC) blood samples obtained before immunoprophylaxis.
- Immunoprophylaxis failure: **4.4 %** (17/385); all born to HBeAg(+) mothers whose HBV-DNA were > 6 log 10copies/mL.
- Only **4/17** with high HBV-DNA at birth; In-utero infection less prevalent than appreciated??



Zhang et al. BMC ID 2016

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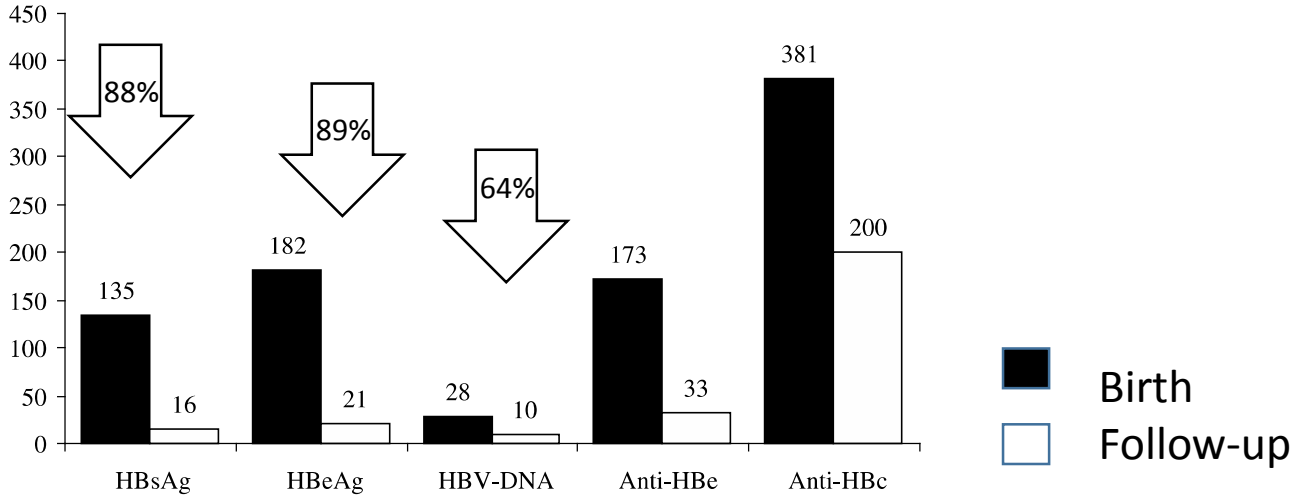
- Only 4/17 with high HBV-DNA at birth; In-utero infection less important than appreciated??
- **HBV markers at birth cannot diagnose or exclude MTCT**



Zhang et al. BMC ID 2016

Is there a marker that may identify HBV infected infants?

- 148 HBsAg(+) mother-infant pairs; 94% HBV genotype C
- Mothers: 27% HBeAg (+), most high HBV-DNA levels
- All babies received combined immunoprophylaxis
- Neonates were found at birth: 28% HBsAg (+);16% HBV-DNA(+) and 24% HBeAg(+)

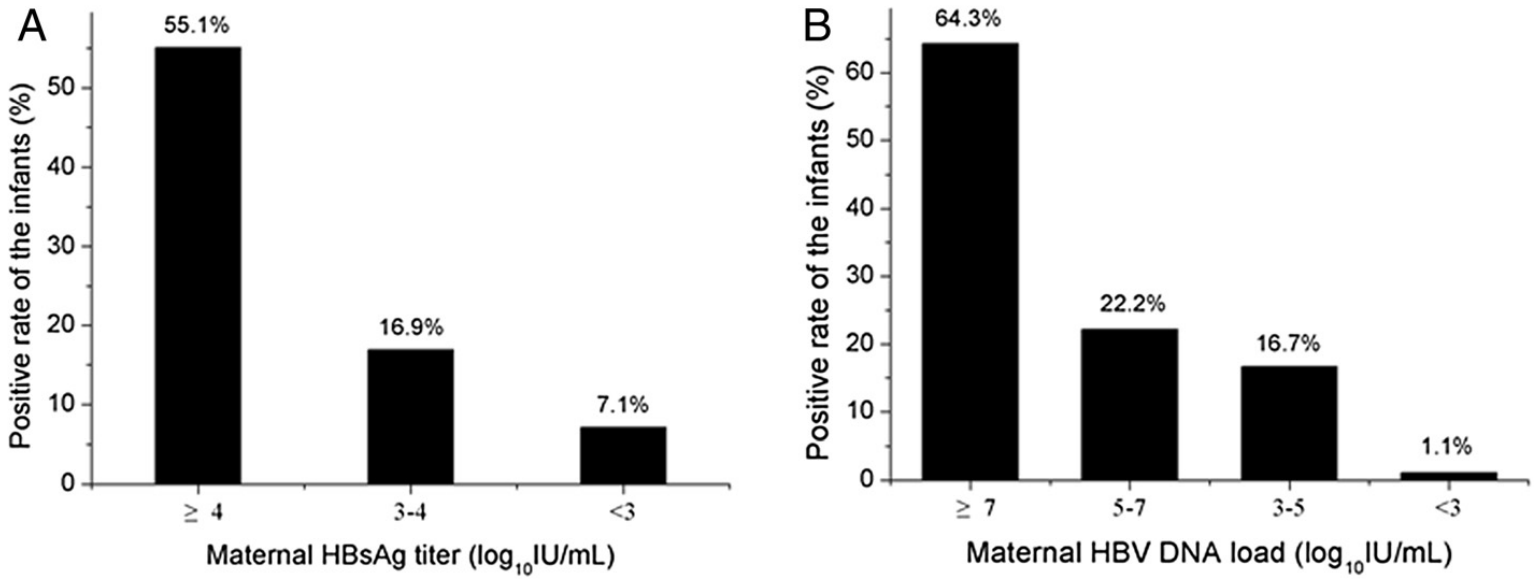


Figure 1 Correlation of HBsAg and HBV DNA between mothers and newborns. HBsAg(+) and HBV DNA(+) rates of the infants at birth in different levels of maternal HBsAg titer **(A)** and HBV DNA load **(B)** groups.

Chen T et al BMC ID 2013

Is there a marker that may identify HBV infected infants?

- 148 HBsAg(+) mother-infant pairs; 94% HBV genotype C
- Mother: 27% HBeAg (+), 26% high HBV-DNA levels
- Neonates were found at birth: 28% HBsAg (+);16% HBV-DNA(+) and 24% HBeAg(+)

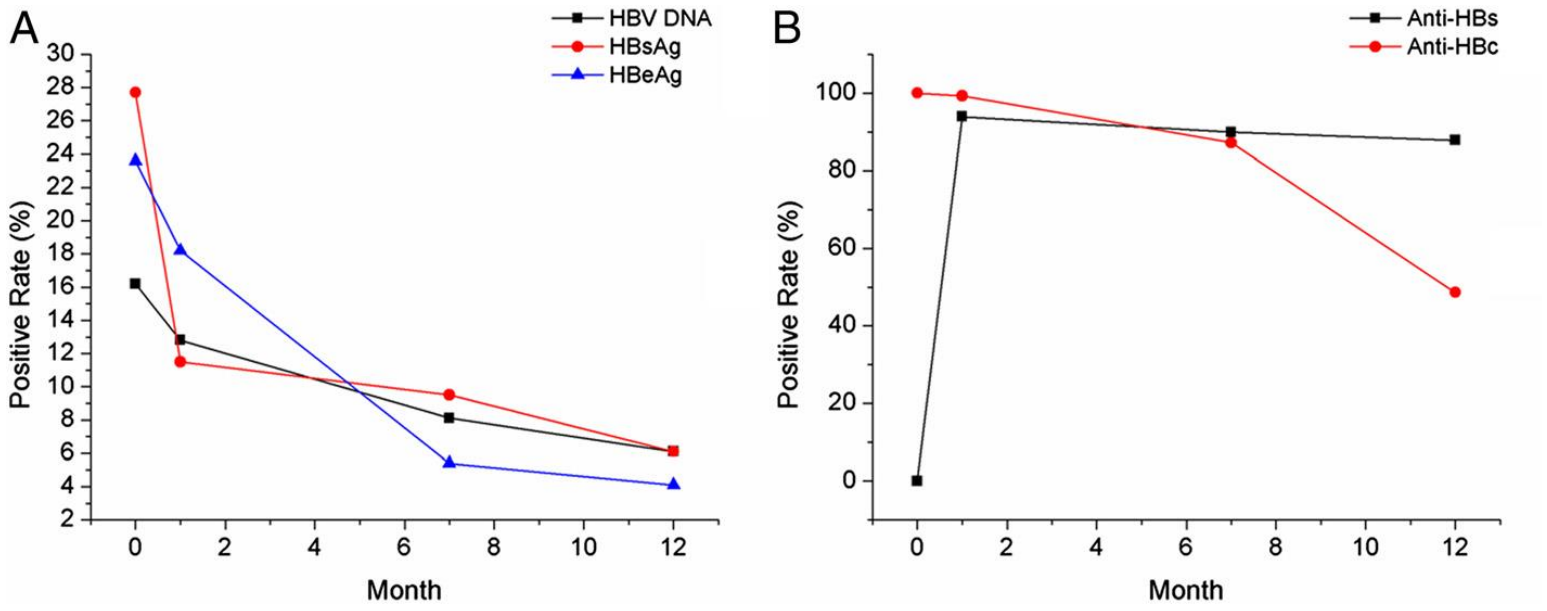


Figure 2 HBV DNA(+), HBsAg(+), HBeAg(+), anti-HBc(+), and anti-HBs(+) rates in infants. Positive rates of **(A)** HBV DNA, HBsAg, and HBeAg and **(B)** anti-HBc and anti-HBs at birth, 1 mo, 7 mo and 12 mo.

Chen T et al BMC ID 2013

Is there a marker that may identify HBV infected infants?

- 148 HBsAg(+) mother-infant pairs; 94% HBV genotype C
- Mother: 27% HBeAg (+), 26% high HBV-DNA levels
- Neonates were found at birth: 28% HBsAg (+);16% HBV-DNA(+) and 24% HBeAg (+)
- **Immunoprophylaxis failure: 9 infants (6.1%)**

Table 3 Positive likelihood ratio of diagnostic indicators for chronic HBV-infected infants

Diagnostic indicators	N	Infected		Uninfected		Positive likelihood ratio
		N	True positive ratio	N	False positive ratio	
HBV DNA(+) at birth	21	9	9/9 = 1	12	12/139 = 0.086	11.6
HBsAg(+) at birth	41	9	9/9 = 1	32	32/139 = 0.230	4.34
HBV DNA- and HBsAg- positive at birth	18	9	9/9 = 1	9	9/139 = 0.065	15.4
Anti-HBs(–) at 1 month old	9	9	9/9 = 1	0	0/139 = 0	+∞

Chen T et al BMC ID 2013

Diagnosis of HBV infection in infants

- **HBsAg (+) infants for > 6months**
- HBsAg / HBV DNA positivity in the cord blood (? contamination)
- HBV seromarkers and HBV DNA in venous blood persist in older infants?

- Some of these infants may represent **occult HBV infection** ?
- Definition: HBsAg (-) and HBV DNA (+)

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Zhu Q, et al. Chin Med J (Engl) 2003

Diagnosis of HBV infection in infants

- **HBsAg (+) infants for > 6months**
- HBsAg / HBV DNA positivity in the cord blood (? contamination)
- HBsAg / HBV DNA in venous blood and persists after the age of >3 months

- Some of these infants represent **occult HBV infection** ?
- Definition: HBsAg (-) and HBV DNA (+)
- **Most infants achieve protective levels of anti-HBs antibodies**
- **Most infants do NOT develop anti-HBc (+) antibodies**

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Zhu Q, et al. Chin Med J (Engl) 2003

Are these infants with true occult HBV infection?
Are they really infected ?

Occult HBV infection in immunized neonates born to HBsAg(+) mothers

Prospectively followed 32 infants diagnosed with OBI at 7 months: HBsAg(-)/anti-HBsAg(+) but HBV-DNA (+)

	12 months (N=32)	24 months (N=32)	36 months (N=26)
HBV DNA (+)	8/32 (**25%**)	7/32 (**22%**)	2/26 (**7.7%**)
Median HBV –DNA level (log IU/mL)	1.81 (1.28–2.91)	1.94 (1.23–2.58)	1.74 (1.59–1.89)
Anti-HBs (+)	30/32 (93.8%)	21/30 (70%)	14/17 (82.4%)
Median anti-HBs (mIU/mL)	239.2 (127.1–450.2)	26.7 (8.4–32.5)	34.3 (17.6–67.1)
Anti-HBc (+)	**22**	**5**	**2**

- **Timely administration of the 1st dose of vaccine within 6 hours of birth** reduced the OBI rate from **38.2%** (13/34) to **15.3%** (19/124) (***p* = 0.003**).
- No correlation with maternal HBeAg (+), HBsAg titers or HBV-DNA levels.
- No vaccine escape mutants found.
- **HBV infection is controlled in immunized infants**

Lu Y et al PLoS ONE 2016

Adequate levels of anti-HBs after vaccine and HBIG immunoprophylaxis eventually may clear the virus

- Prospectively followed 17/21 children with documented occult HBV infection post passive-active immunoprophylaxis
- At mean age **6.57** ± 2.75 years:
  - All remained HBsAg (-)
  - **16/17 were HBV DNA (-)**
  - Two children developed anti-HBc antibodies
  - One child remained HBVDNA(+) with low viremia (50 copy/mL), carried the G145R mutation

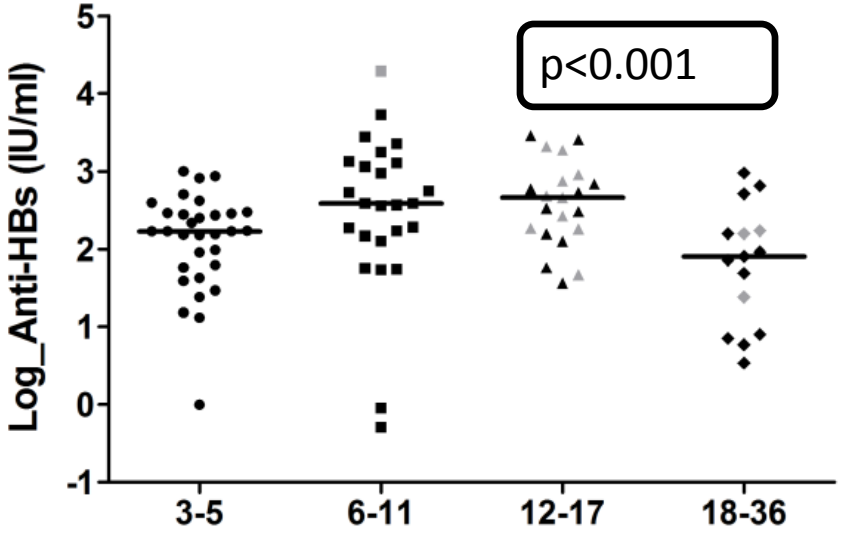
Pande et al reported that development of anti-HBs >10 IU/mL at 18weeks of age was associated with clearance of occult HBV infection

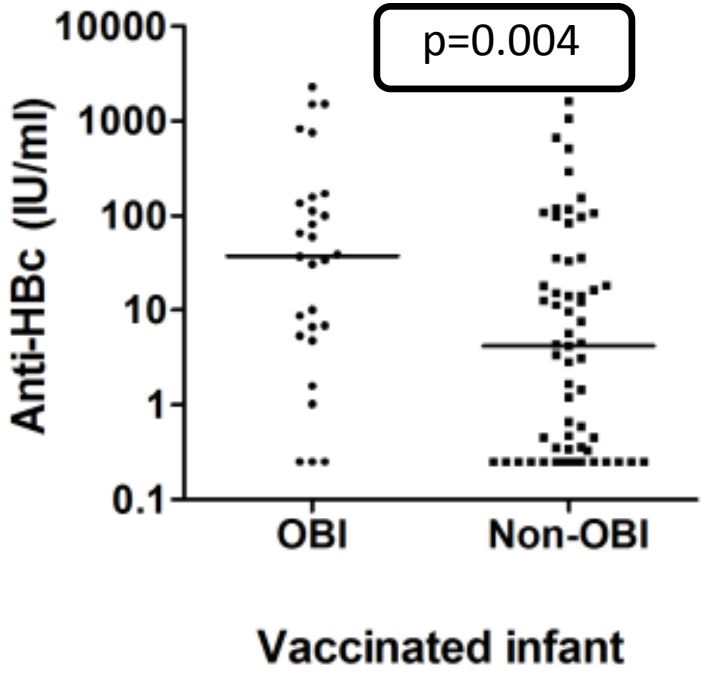
Sadeghi A et al. JVH 2016
Pande C et al JVH 2013

"Transient" occult HBV infection in immunized infants born to HBsAg(+) mothers

- HBsAg was detected in 3/77 (**3.9%)** babies.
- HBV DNA was detected in 28/77 (**36.4%**) HBsAg(-) infants
- **The frequency of OBI decreased with age**:
  - 48.4% <6 months to 18.2% at ≥12 months of age (p=0.06).

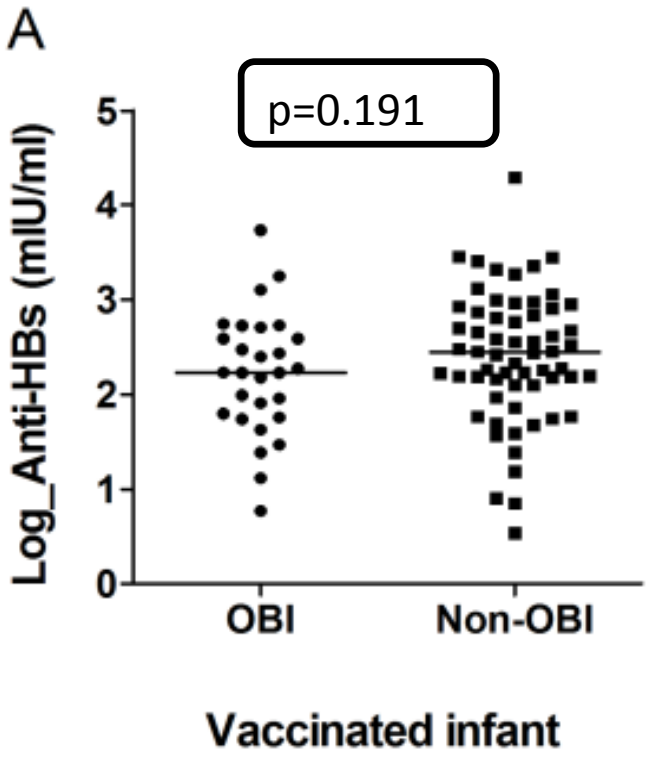
A

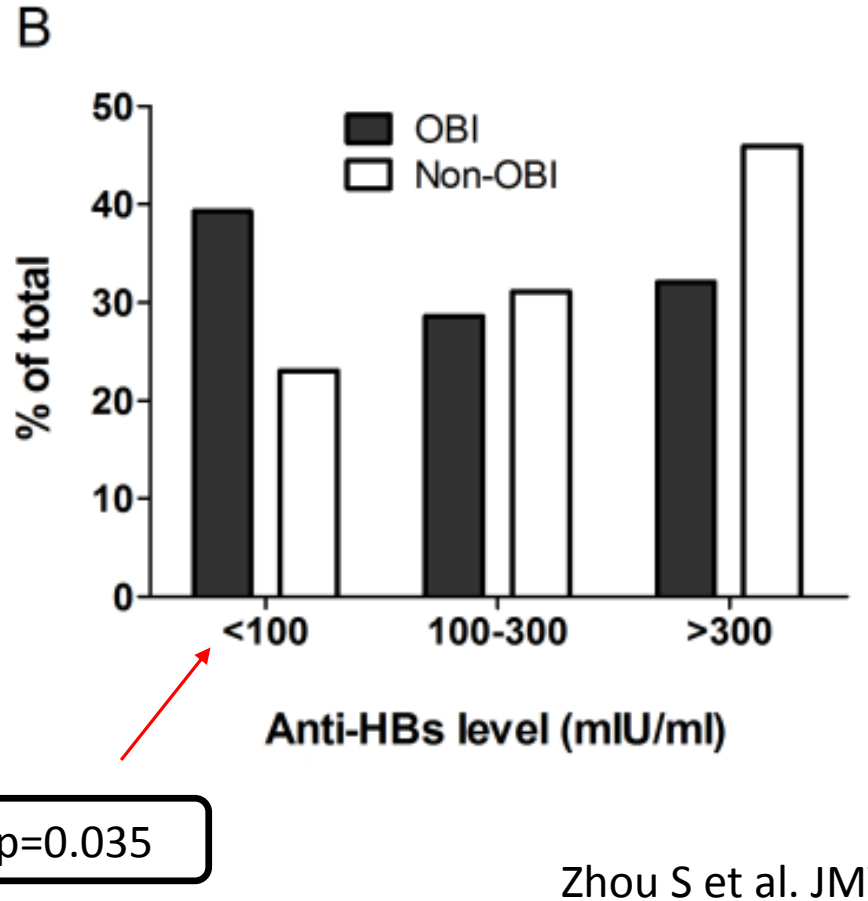




Zhou S et al. JMV May 2017

"**Transient**" occult HBV infection in immunized infants born to HBsAg(+) mothers





Zhou S et al. JMV May 2017

Population based study assessing OBI prevalence in <18yo

Table 3. Estimated Rates of OBI in HBsAg-Negative Subjects With or Without Infant Hepatitis B Immunization

	Unvaccinated Cohorts	Vaccinated Cohorts
Prevalence rate of specific HBV marker profile in total enrolled children population in previous serosurveys, %		
HBsAg (+) rate*	9.8	0.5
Anti-HBc (+) rate†	26.2	2.9
HBsAg (–) but anti-HBc (+) rate‡	16.4	2.4
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Prevalence rate of OBI in children with or without anti-HBc positivity who were sampled for analysis in the present study		
Rate of OBI in HBsAg (–) but anti-HBc (+) subjects‖, %	1.7	4.8
Rate of OBI in HBsAg (–) but anti-HBc (–) subjects¶, %	1.8	0
Estimated frequency of OBI per 10^4 HBsAg-negative children#	160.7	11.5

Hsu HY et al Hepatology 2014

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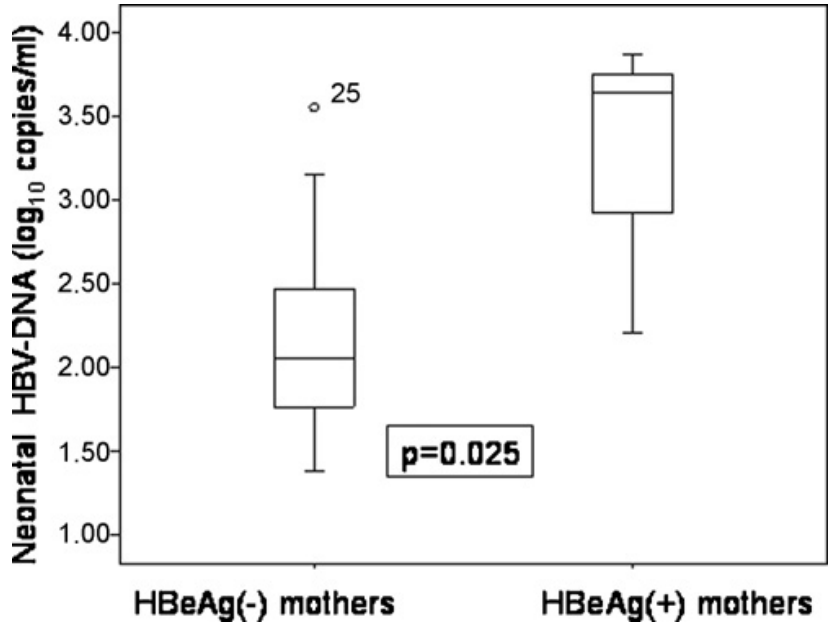
Conclusions

- **Breakthrough infections in immunized subjects seem to result in OBI while in unvaccinated subjects natural infection will ensue.**
- In the postvaccination era, the presence of **anti-HBc** is a very useful marker for OBI screening in HBsAg-negative subjects.
- HBsAg(-) due to a very-low-level viral replication and HBsAg expression ?

Hsu HY et al Hepatology 2014

HBV viremia in newborns of HBsAg(+) predominantly **Caucasian HBeAg(–) mothers**

- HBV-DNA detected in 73.4% of the mothers (93% HBeAg-).
- **HBV-DNA (+) detected in 27/109 (24.7%) newborns**
  - 3/8 (**37.5%**) of HBeAg(+) mothers
  - 24/101 (**23.8%**) of HBeAg(-) mothers (p=0.39)
- No association with maternal viremia or maternal VL
- No association with mode of delivery
- Association between maternal HbeAg status and level of neonatal viremia.



Papaevangelou V et al JCV 2011

HBV viremia in newborns of HBsAg(+) predominantly **Caucasian HBeAg(–) mothers**

Upon follow-up

- At 9 months of age:
  - all children were HBsAg(-)
  - 97.2% had developed anti-HBs antibodies
- At 24 months of age:
  - all OBI re-examined were HBV DNA (-);
  - one child had developed anti-HBc antibodies

Papaevangelou V et al JCV 2011

What is the pathogenesis of HBV-DNA (other seromarkers) detection in infants?

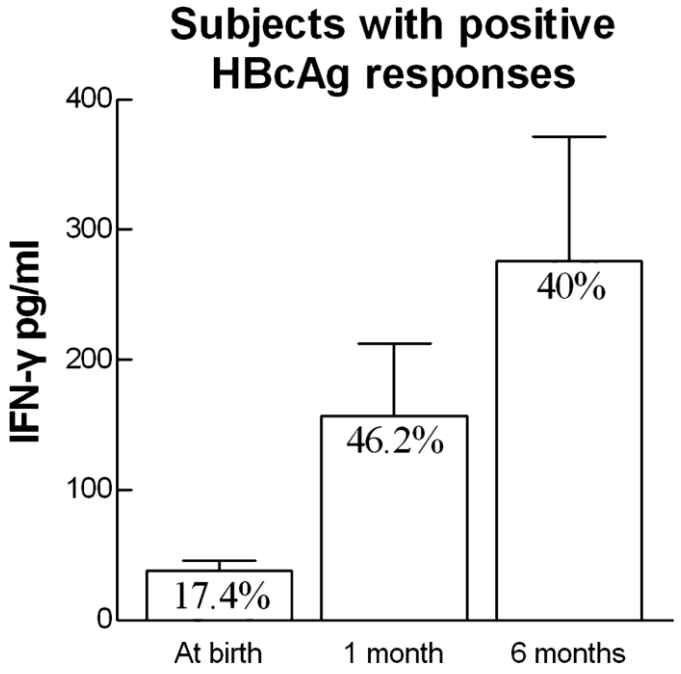
- Using high sensitivity real time PCR, we are able to detect low levels of viremia that do not cause infection?
- Placenta leakage of maternal non-infectious antigens (Dane particles)?
- Maternally derived HBV infected PBMCs transferred ?
- Perinatal transmission that is cleared by the infant post prophylaxis?
- HBV cccDNA long persists in hepatocytes, resulting in intermittent viraemia ?

Shao Q et al. Arch Gynecol Obstet 2013

Are there any implications of the HBV-DNA detection in infants?

- Clinical significance not clear
- Infants develop adaptive cell mediated immunity
- Few children with persistent OBI

- Responses to vaccination ?
  - neonatal HBV viremia in HBsAg(−) infants is clinically important has been associated with vaccination failure



Koumbi L et al. Cell Mol Immunol 2010
Badur S et al. JID 1994
Shi I et al ZEKZZ 2006

Conclusions

- Need to better differentiate:
  - Immunoprophylaxis failure (HBV infection)
  - Occult HBV infection (HBsAg-/HBV-DNA+/anti-HBc)
  - Transient HBV-DNA viremia

- Most likely these 3 outcomes are:
  - “exposure dependent” or
  - “infant immune response dependent” ? the role of HBIG?
  - different stages of HBV infection ?

Thank you for your attention

Backup slides

Administration of HBIG and occult infection

- Randomized 259 newborns to receive vaccine versus vaccine + HBIG
- 81% of mothers HBeAg(-)
- At 18 weeks of age 64% infants had OBI infection (HBsAg-/HBV-DNA+)
- OBI significantly more common in the HBIG group :
  - 76/106 (72%) versus 66/116 (57%); p = 0.025.
- Development of anti-HBs (+) at 18 weeks of age was associated with clearence of HBV-DNA in babies with occult HBV infection.
- At 24 months of age 42% infants had OBI infection

Pande et al. JVH 2013

Population based study assessing OBI prevalence in <18yo

Hsu HY et al Hepatology 2014

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Conclusions

- **Breakthrough infections in immunized subjects seem to result in OBI while in unvaccinated subjects natural infection will ensue.**
- In the postvaccination era, the presence of **anti-HBc** is a very useful marker for OBI screening in HBsAg-negative subjects.
- A very-low-level viral replication and HBsAg expression, rather than surface gene mutations that may escape detection by HBsAg screening assays, is the major mechanism related to OBI.

Hsu HY et al Hepatology 2014

The role of HBeAg

- Transplacental maternal HBeAg may induce immunologic tolerance in utero, thereby facilitating MTCT of HBV infection.
- HBeAg induces specific unresponsiveness of helper T cells to HBcAg and HBeAg in the neonates born to HBeAg (+) mothers
- Therefore not only transmission but also chronicity rates of perinatal HBV infection increase:
  - 28.2% (17.4%–33.9%) in infants born to HBeAg(-) mothers
  - 64.5% (53.5%–100%) in infants born to HBeAg (+) mothers.

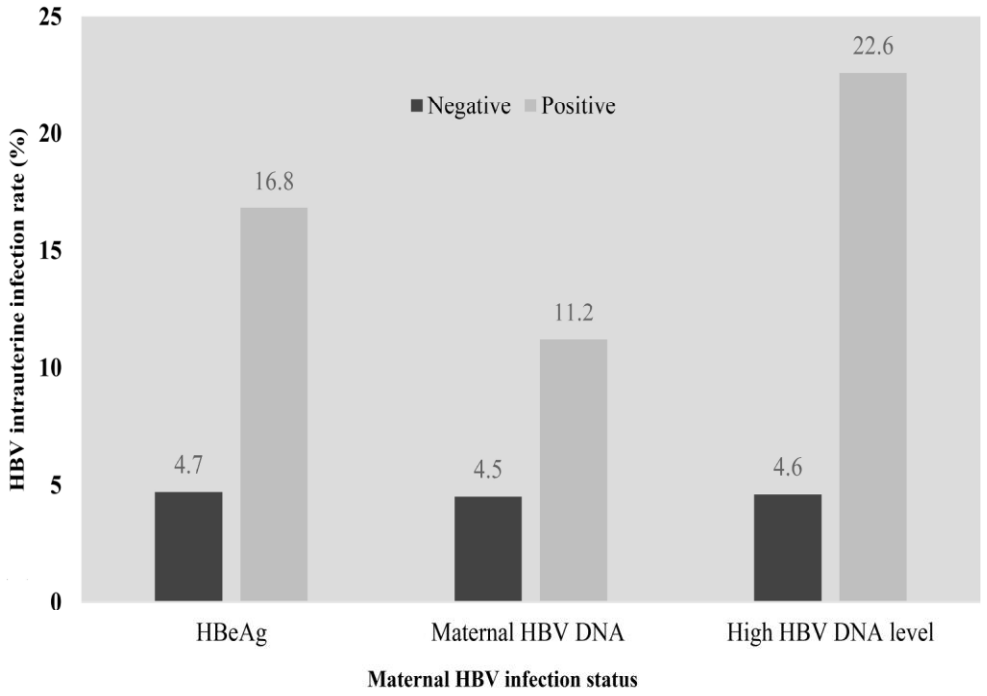
Milich DR, et al. Proc Natl Acad Sci 1990
ZX Li et al. Emerging Microbes and Infections 2015.

Maternal viremia

- **Elesfsiniotis1:** Overall, 1.156% of women were HBsAg(+) and the majority of them (71.3%) were Albanian. The prevalence of HBsAg was 5.1% in Albanian women, 4.2% in Asian women and 1.14% in women from Eastern European countries. The prevalence of HBsAg in African (0.36%) and Greek women (0.29%) was very low. Only 4.45% of HBsAg (+) women were also HBeAg(+) whereas the vast majority of them were HBeAg(-)/anti-HBe(+). Undetectable levels of viremia (<200 copies/mL) were observed in 32.26% of pregnant women at labor and 29.03% exhibited extremely low levels of viral replication (<400 copies/mL). Only two pregnant women exhibited extremely high serum HBV- DNA levels (>10 000 000 copies/mL), whereas 32.26% ex hibited HBV-DNA levels between 1 500 and 40 000 copies/mL.
- **Elesfsiniotis2:**SeroprevalenceofHBsAgin26,746womenatreproductiveageinGreeceandevaluationofHBeAg/anti-HBeserologicalstatusas well as serum HBV–DNA levels in a subgroup of HBsAg(+) women at labor. Only 2.67% of HBsAg(+) women were HBeAg(+). Of a subgroup of women in labor with available serum samples 28.6% had undetectable levels of viremia (<200 copies/ml) and 15.9% had extremely low levels of viral replication (<400 copies/ml). Only 12.7% of pregnant women evaluated at labor exhibited extremely high serum HBV–DNA levels (>10,000,000 copies/ml) whereas 42.8% of them exhibited HBV–DNA levels between 1500 and 40,000 copies/ml.
- **Conclusions:** The HBeAg( )/antiHBe(+) serological status is a finding observed in the vast majority of HBsAg(+) women of our study population, and a significant percentage of them (approximately 44.5%) exhibit extremely low or even undetectable levels of viral replication at labor, suggesting possibly that only a proportion of HBsAg(+) women in Greece exhibit an extremely high risk of vertical transmission of the infection.

- Despite the predominance of HBeAg-negative serological status, about one- third exhibit significant (>10 000 copies/mL) or even extremely high (>10 000 000 copies/mL) viral replication levels at perinatal period,4 basically due to precore mutation **(G1896A)** of the HBV genome, a mutation that is frequently observed in the Mediterranean basin.5

HBV intrauterine infection rates



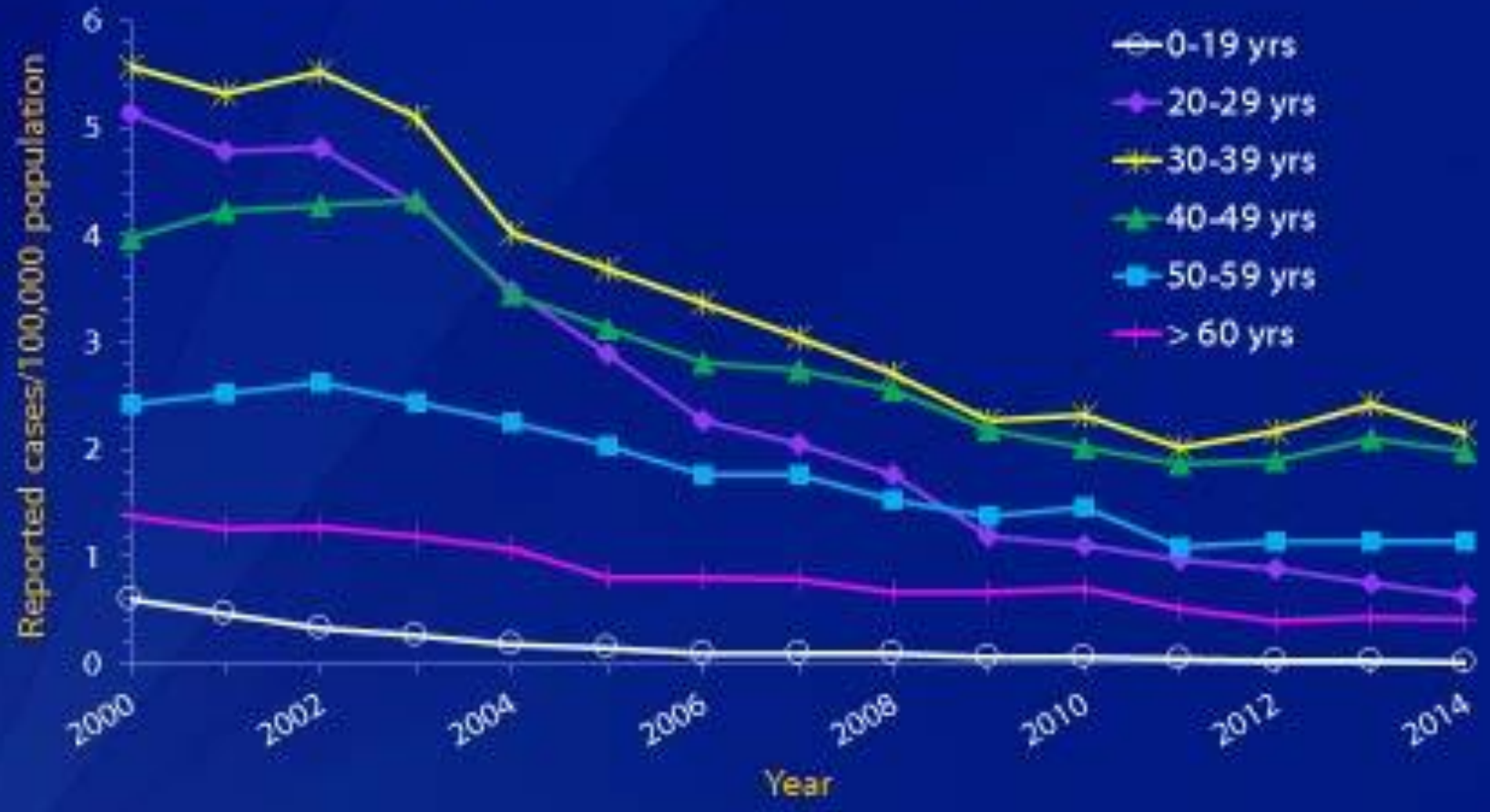
Songxu Peng et al
JGH_2017_under review

Fig.1. HBV intrauterine infection rates of the infants born to mothers with different characteristics.

Outline

- Epidemiology/ evidence of viremia – studies
- How it happens – risk factors (genotype?, cs?)
- Why transient – evidence
- Innocent?
  - Association with infection
  - Association with immune response to vaccine
  - Implications for use of HBIG
  - Implications for deferred - delayed HBV vaccination
- Future research

Incidence of Acute Hepatitis B, by Age Group — United States, 2000-2014



National Notifiable Diseases Surveillance System (NNDSS)

ACIP 2016

What is the role of HBsAg escape variants?

- HBsAg variants do not play a major role in OBI pathogenesis.
- “Breakthrough infections caused by S-gene mutants are occasionally reported but do not pose a serious threat to the established vaccination programs.”

Zhang et al. Virology Journal 2016
Romano et al HVI 2015
Shahmoradi S et al J Hepatology 2012