Maternal oral CMV recurrence following postnatal primary infection in infants

No conflict of interest
CMV seroprevalence among United States women of reproductive age

≈60% seropositive women
Non primary CMV infection

= CMV Recurrence

**REINFECTION**

- Antigenic diversity of the virus
- 15 to 90% mixed-genotype infections

**REACTIVATION**

*Latency*: undifferentiated progenitor cells in the bone marrow

**Excretion in urine, breast milk, cervical secretion, saliva...**

**No symptoms in immunocompetent hosts**
Cervical and urinary excretion

- 659 pregnant women; % of positive culture
  - 9% in cervix
  - 5% in urine
  - Risks factors: primiparity, young age

Stagno S. J Infect Dis. 1975 May;131(5):522-7

- 134 women attending a STD clinic in Seattle
  - 34% of women older than 21 years of age had shed CMV in the cervix
  - many had evidence of shedding of multiple strains

Chandler SH. J Infect Dis 1987; 155:655-660
Risk of CMV congenital infection

Kenneson 2007, de Vries 2013
CMV postnatal transmission

- mother-to-child via breastfeeding,
- parents- or siblings-to-child via close contact,
- child-to-child via close contact in out-of-home settings such as day care centers.
- intra-partum transmission
Postnatal CMV primary infection in newborns

- Prospective study of 539 very low birth weight infants (birth weight <1500 grams): 7% incidence of postnatal CMV infection at 12 weeks postnatal age
  
  Josephson CD, 2014

- Vulnerable to severe CMV disease:
  - 15% sepsis-like syndrome (hepatosplenomegaly, pneumonitis, and abnormalities of blood counts and liver function tests)
  - Necrotizing enterocolitis
  - ?Long term impact

Gunkel J, 2014; Brecht KF, 2015
96% of CMV recurrence in breastfeeding mothers

DNA in milk cells

Proportion

Time after delivery (days)

Transmitters

Non-transmitters

Hamprecht, 2001
Objective

To examine the pattern of CMV oral recurrence in postpartum women in relation with infant postnatal primary infection in a well-characterized prospective cohort of households of mothers and children in Uganda.
Goal: to comprehensively characterize primary HHV infections in a high-prevalence population with respect to:

1. Incidence
2. Risk factors for acquisition
3. Viral replication patterns
4. Viral genomic diversity
5. Immune control
PHICS design

- Enrolled pregnant women with >1 child <7 years old at home and documented HIV status in Kampala, Uganda

- Followed the mother, newborn child, and siblings

- At each *weekly* household visit:
  - Oral swab collected from all subjects for HHV qPCR
  - Breastfeeding, and saliva sharing behaviors documented
  - *Able to measure exposures*

- Blood collected every 4 months in primary infant and annually in mothers

2008-2010
Techniques

- **Real-time quantitative (q)PCR:**
  - limit of detection 150 copies/mL.

- **Serology:** ELISA kits (Wampole (Alere), Boucherville, Quebec).

- **Type specific serology:**
  - polymorphisms in antibody binding sites within envelope glycoproteins gH and gB
Definitions

- **Infant primary infection**: (q)PCR+ in the plasma or $\geq 3.5 \log_{10}$ copies/mL in $\geq 2$ consecutive oropharyngeal swabs
  - time of primary infection = date of the earliest positive PCR

- **Maternal recurrence**: (q)PCR $\geq 3 \log_{10}$ copies/mL in $\geq 2$ consecutive oropharyngeal swabs,
  - Onset = time of the first positive swab that followed two negative swabs.
  - End = first series of 2 negative swabs.
Analyses

• Bivariate analyses

• Generalized estimating equations using time-depended variables
Results

32 mother-infant pairs

30 households
Follow-up: median = 57 weeks (range 3 to 119)
28 siblings

2 cases of congenital infections

All women CMV IgG+

17 women HIV+ on antiretrovirals
→ No cases of HIV MTC transmission
30 households
Follow-up: median = 57 weeks (range 3 to 119)
28 siblings

15 women with CMV oral recurrence
Median onset: 15 weeks after delivery (range 6 to 43)
Median duration: 18 weeks (range 4 to 42)

20 cases of infant postnatal primary infection
4 not breastfeed within 3 weeks before the infection.

28 siblings
Infant infection day = dotted line; Maternal episode start day = red line

Days after infant birth

Log\textsubscript{10} CMV copies per swab
Time to maternal oral recurrence following infant primary infection

Median: 6 weeks (range 1 to 10) after infant primary infection
<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Maternal recurrence n=15</th>
<th>No maternal recurrence n=15</th>
<th>Odds ratio (p-value)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive status</td>
<td>14</td>
<td>8 (53%)</td>
<td>6 (43%)</td>
<td>1.5 (0.715)</td>
</tr>
<tr>
<td>Breastfeeding (ever)</td>
<td>25</td>
<td>14 (93%)</td>
<td>11 (79%)</td>
<td>3.65 (0.33)</td>
</tr>
<tr>
<td>Breastfeeding (associated with infant infection)</td>
<td>14</td>
<td>13 (87%)</td>
<td>1 (50%)</td>
<td>5.51 (0.331)</td>
</tr>
<tr>
<td>Saliva sharing ever</td>
<td>12</td>
<td>6 (40%)</td>
<td>6 (43%)</td>
<td>0.89 (1)</td>
</tr>
<tr>
<td>Food chewing ever</td>
<td>12</td>
<td>4 (27%)</td>
<td>8 (57%)</td>
<td>0.29 (0.139)</td>
</tr>
<tr>
<td>Postnatal infant CMV primary infection</td>
<td>17</td>
<td>15 (100%)</td>
<td>2 (14%)</td>
<td>Inf (0)</td>
</tr>
</tbody>
</table>
Household viral shedding (primary infant and siblings) according to maternal CMV recurrence status
# Multivariate final model

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Odds ratio (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding</td>
<td>3.8 (0.079)</td>
</tr>
<tr>
<td>Food chewing behavior in the previous week</td>
<td>5.2 (0.071)</td>
</tr>
<tr>
<td>Infant viral shedding the previous week (log10 CMV DNA copies/swab)</td>
<td>2.6 (&lt; 0.001)</td>
</tr>
</tbody>
</table>
Dose-response relationship between primary infant viral concentration in saliva in a given week to mother’s recurrence status in the following week.
CMV reinfection?

No recurrence

- 50%
- 50%

Recurrence

- 22%
- 78%

- Reinfection
- No reinfection
Summary

- N=30 mother-infant-sibling cohort
- Maternal CMV oral recurrence: 50%
  - always occurred after infant primary CMV infection
Hypothesis 1: Differential shedding by compartments

- CMV virus diseminate to a wide range of host tissue compartments and cell types
- No correlation between CMV shedding in the genital tract and urine nor between breast milk and saliva

Postpartum women could shed CMV in breast milk before than in saliva, allowing for the transmission of CMV to newborns before maternal oral recurrence.
Hypothesis 2: Compartmentalized shedding of different strains

- CMV is genetically diverse within each host

Mothers could infect newborns through breast milk and then newborns reinfec
the same virus.
Hypotheses 1 and 2

• Supported by
  ▫ the association between breastfeeding and postnatal CMV primary infection
  ▫ the lack of association between maternal recurrence and reinfection

• Moreover: onset of CMV shedding in breast milk within the first 2 weeks after delivery

Hypothesis 3: Infection of the newborn by another source

Then mothers could have recurrence because of reinfection through the exposure to new CMV strains originating from the newborns.

• But:
  ▫ Association between breastfeeding and postnatal CMV primary infection.
  ▫ No relationship between CMV shedding in siblings and maternal recurrence.

Limits

- Missing data on CMV shedding in breast milk and on other potential sources of CMV exposure.

- Sample size limits our ability to assess the association between shedding and postnatal primary infection according to feeding practice.

- Interpretation of data on maternal reinfection: type-specific serology assay limited to 4 CMV strains likely not detect all reinfections.
Conclusion

• Temporal association between infant postnatal CMV primary infection and maternal oral recurrence: statistically significant occurrence of maternal oral recurrence AFTER infant primary infection

• Likely a practical illustration of CMV compartmentalization and/or heterogeneity within each host.
Thank you
Supplementary slides
Median CMV oral shedding (log10 copies/swab) in siblings according to newborn CMV status (primary postnatal infection) and feeding practice

![Box plot showing median CMV oral shedding in siblings]

- Not infected (n=10)
- Infected (breastfed) (n=14)
- Infected (no breastfed) (n=3)