Safety and Tolerability following Consumption of *Bifidobacterium longum* subspecies *infantis* in exclusively breastfed term infants: IMPRINT Study

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Disclosures
Benefits of breastfeeding no matter $\$

**Infant**
- Reductions in child infections
- Reductions malocclusion
- Increases in IQ
- Reductions in overweight and diabetes

**Mother**
- Protection against breast and ovarian cancers
- Protection against type 2 diabetes
- Improved birth spacing

**Overall**
- BF universally prevent 823,000 annual deaths in children < 5 years and 20,000 annual deaths from breast cancer

Human milk oligosaccharides

- Highly variable
- 200 species
- Indigestible
- Nourishment
- Intestinal

Bifidobacterium

Nature 468 S5-S7 (23 December 2010)
Garrido et al Microbiology (2013)
Gut microbiome: infants

The diagram shows the changes in the gut microbiome over chronological age (mo) in infants, with population-survey weighted relative abundances. The x-axis represents chronological age in months, ranging from 0 to 24. The y-axis represents the relative abundance of different bacterial species, with categories for maximum relative abundance and below detectable abundance.

Key species and their abundances over time include:
- Staphylococcus sp.
- Streptococcus sp.
- Bifidobacterium sp. (putative breve)
- Bifidobacterium longum
- Lactobacillus ruminis
- Dorea longicatena
- Dorea formicigenerans
- Bifidobacterium sp. (putative catelunatum)
- Faecalibacterium prausnitzii
- Ruminococcus species

The diagram illustrates the transition of the gut microbiome from a milk-oriented microbiota to a more complex composition as infants grow older.
Effect of HMOs on *B. infantis*

*B. infantis* grows on HMOs

LoCascio et al., JAFC 2007
**B. infantis** HMO gene cluster

- HMOs are bound by SBP lipoproteins proximal to permeases
- ATP hydrolysis prompts transport of oligosaccharides across membrane
- Intracellular glycolytic enzymes deconstruct oligosaccharide

Sela PNAS 2008
Effect of HMOs on *B. infantis*

- Enhance binding to intestinal cells
- Induce expression of intestinal tight junction proteins
- Induce expression anti-inflammatory cytokines (IL-10)
- SCFA production
### Developed Countries: infant gut dysbiosis

<table>
<thead>
<tr>
<th>Country (Cohort Type)</th>
<th>Average % of Infant Fecal Microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh (Vitamin A supplement)</td>
<td>90% Actinobacteria, 10% Firmicutes</td>
</tr>
<tr>
<td>Gambia (Random)</td>
<td>80% Bacteroidetes, 20% Firmicutes</td>
</tr>
<tr>
<td>Armenia (Random)</td>
<td>95% Firmicutes, 5% Proteobacteria</td>
</tr>
<tr>
<td>Switzerland (Random, values estimated from graph)</td>
<td>70% Firmicutes, 30% Other</td>
</tr>
<tr>
<td>Canada (Random, but at 4 months of age)</td>
<td>85% Firmicutes, 15% Proteobacteria</td>
</tr>
<tr>
<td>Georgia (Random)</td>
<td>90% Firmicutes, 10% Others</td>
</tr>
<tr>
<td>Sweden (Healthy)</td>
<td>95% Firmicutes, 5% Proteobacteria</td>
</tr>
<tr>
<td>Italy (Colicky infants)</td>
<td>75% Firmicutes, 25% Proteobacteria</td>
</tr>
<tr>
<td>Davis, CA, USA (Non-secretor mother enriched cohort)</td>
<td>80% Firmicutes, 20% Proteobacteria</td>
</tr>
<tr>
<td>Ireland (Healthy, some formula feeding)</td>
<td>90% Actinobacteria, 10% Bacteroidetes</td>
</tr>
</tbody>
</table>

Graph showing distribution of microbial types with labels for anti-inflammatory and pro-inflammatory categories.
C-sections
- Food allergy/atopy, allergic rhinitis, asthma, hospitalization for asthma OR = 1.18-1.32 (0-31 y)³
- Wheezing and food allergy OR = 1.2-1.64 (2 y)⁴

Antibiotic exposure in infancy
- Use in first 2 y dose-dependently increase asthma, eczema, hay fever by age 7.5 y (asthma: OR=1.1, 1.5, 1.8, 2.8)⁵
- Use in first 1 y increase asthma OR= 2.0 (1-18 y)⁶

Formula feeding
- Exclusive BF first 3 months of life protective against asthma OR= 0.70 (1-8 y)⁶
- Exclusive BF first 3 months of life protective against allergic rhinitis OR= 0.74 (1-4 y)⁶
Infant Microbiota and Probiotic Intake (IMPRINT) Study
Objective

Determine if exogenous delivery of an activated *Bifidobacterium longum* subsp. *infantis* for 21 days to exclusively breastfed infants is tolerable and safe and increases intestinal *B. infantis* during and 1 month post-supplementation.
Study Design

**Inclusion**
- Healthy mothers, 21-45 yr, Yolo/Sacramento
- Healthy term infants >37 wk;
- Intention to exclusively breastfeed for 3 mos.

**Exclusion**
- Plan to administer probiotics to infants
- Infants on antibiotics after 72 h of age
- Separate discharge for more than 24 hours
Study Design

- Parallel randomized control trial, UC Davis IRB
- Randomized lactation support (LS, n = 34) or *B. infantis* + lactation support (BiLS, n = 34)
  - C-section matched for time of ruptured membranes: (≤ 6 h or >6 h)
- Primary outcome variable: fecal *Bifidobacterium* (fold-difference 1.3, α=0.05, β=0.9) and GI tolerability (n = 30)
- *B. infantis* dose provided in one 625 mg sachet 1×10^10 CFU + lactose (Day 7-Day 27)
- Dispensed by UC Davis IDS

Lewis et al. Microbiome, 2015
Hoy-Schulz et al., BMC Complementary and Alternative Medicine, 2016
Safety and Tolerability Data

- **Fecal *Bifidobacterium***
  - RT PCR, Evolve Biosystems Inc.

- **Daily feeding logs**
  - # BF, formula, liquids, solids vitamins, supplements, probiotics, *B. infantis*

- **Daily GI & health logs**
  - # Stools, consistency modified Amsterdam scale, blood in stool, body temperature, frequency spit-ups (<5, 5-10, >10), flatulence (never, sometimes, often, very often), intake of antibiotics, medications
  - Ratings of GI-related symptoms using a continuous scale 0 (“not noticeable”) to 10 (“most severe”): 1) general irritability (“how irritable was your baby?”), 2) upset (“if your baby vomited or spit up, how upset was he/she after?”), 3) and discomfort (“rate your baby’s discomfort in passing stool or gas”)

- **Weekly and Biweekly Questionnaire**
  - Episodes of colic, eczema diagnosis # of sick doctor visits; illnesses

- **End of Study Questionnaire**

- **Compliance: D22 and D33**

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Penders et al. EMS Microbiology Letters 2005
Bekkali et al., Journal of Pediatrics, 2009
Statistics

- Intent to treat post Day 7
- Data binned:
  - Daily logs: baseline (D1-6), intervention (D7-27), post-intervention (D28-61)
  - Questions: baseline (D7), intervention (D15, 22, 33), post-intervention (D61)
  - # D reported/total # D OR # infants/total # infants
- IBM SPSS Statistics version 24, GraphPad PRISM v.7. Statistical significance was considered as $P<0.05$.
- Normality: histograms and q-q plots, Shapiro-Wilk test and Levene’s statistic. Log10 transformed.
- Categorical data: Pearson Chi-square Test for Independence, Continuous data: Mann-Whitney U Test, repeated measures ANOVA; ANCOVA with parity covariate, logistic regression for group differences in stool consistency, flatulence and spitting-up.
**Results: Enrollment**

- **Enrollment**
  - Assessed for eligibility (n = 108)
  - Excluded (n = 28)
    - Not meeting Final inclusion criteria (n = 27)
    - Refused to participate (n = 1)
    - Other reasons (n = 0)
  - Non-randomized (n = 15)
  - Randomized (n = 65)

- **Allocation**
  - Allocated to BiLS (n = 41)
    - Received allocated intervention (n = 34)
    - Did not receive allocated intervention (n = 7) (screen-failed post-randomization or discontinued before intervention period initiated)
  - Allocated to LS (n = 39)
    - Received allocated intervention (n = 34)
    - Did not receive allocated intervention (n = 5)

- **Follow up**
  - Lost to follow up (n = 0)
  - Discontinued intervention (n = 0)

- **Analysis**
  - Analyzed (n = 34)
  - Excluded from analysis (n = 0)

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  - Analyzed (n = 34)
  - Excluded from analysis (n = 0)
## Results: Maternal Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>BiLS (n = 34)</th>
<th>Mean</th>
<th>SD</th>
<th>LS  (n = 34)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Enrollment (yr)</td>
<td></td>
<td>33.3</td>
<td>4.5</td>
<td>31.4</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Pre-Pregnancy BMI&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>25.6</td>
<td>3.6</td>
<td>23.8</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Pregnancy Weight Gain (kg)</td>
<td></td>
<td>15.0</td>
<td>5.1</td>
<td>15.2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Hours in Labor (hr)</td>
<td></td>
<td>12.9</td>
<td>12.8</td>
<td>17.8</td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>Ruptured Membranes Prior to Birth (hr)</td>
<td></td>
<td>10.1</td>
<td>17.2</td>
<td>10.8</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>Number of Live Births&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>2.0</td>
<td>1.0</td>
<td>1.4</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Parity, % (n)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td></td>
<td>41%</td>
<td>(14)</td>
<td>76%</td>
<td>(26)</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td></td>
<td>59%</td>
<td>(20)</td>
<td>24%</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Mode of Delivery, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td></td>
<td>68%</td>
<td>(23)</td>
<td>71%</td>
<td>(24)</td>
<td></td>
</tr>
<tr>
<td>C-section</td>
<td></td>
<td>32%</td>
<td>(11)</td>
<td>29%</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Antibiotic Use Labor, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>44%</td>
<td>(15)</td>
<td>29%</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>56%</td>
<td>(19)</td>
<td>71%</td>
<td>(24)</td>
<td></td>
</tr>
<tr>
<td>Labor Complications Reported, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>26%</td>
<td>(9)</td>
<td>26%</td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>74%</td>
<td>(25)</td>
<td>74%</td>
<td>(25)</td>
<td></td>
</tr>
</tbody>
</table>
## Results: Infant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>BiLS (n = 34)</th>
<th></th>
<th>LS (n = 34)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Gestational Age (wk)</td>
<td>39.5</td>
<td>1.2</td>
<td>39.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>3457.8</td>
<td>369.5</td>
<td>3555.6</td>
<td>624.1</td>
</tr>
<tr>
<td>Infant Birth Length (cm)</td>
<td>50.5</td>
<td>2.0</td>
<td>50.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Infant Gender, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62%</td>
<td>(21)</td>
<td>44%</td>
<td>(15)</td>
</tr>
<tr>
<td>Female</td>
<td>38%</td>
<td>(13)</td>
<td>56%</td>
<td>(19)</td>
</tr>
<tr>
<td>Oral or IV Antibiotics 72 hr, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0%</td>
<td>(0 )</td>
<td>3%</td>
<td>(1 )</td>
</tr>
<tr>
<td>No</td>
<td>100%</td>
<td>(34)</td>
<td>97%</td>
<td>(33)</td>
</tr>
</tbody>
</table>
Results: Infant Fecal *Bifidobacterium*

![Graph showing the comparison of Log_{10} CFU Bifidobacterium per gram stool between LS Day 10 and BiLS Day 10. The graph indicates a significant difference (P<0.0001).]
Results: Infant Weight
Results: Infant Feeding

Breast milk intake (#/d)

Baseline | Intervention | Post-intervention
Results: Infant Stooling

Infant Stools (#/d)

Baseline          Intervention    Post-intervention

(P<0.0005)
Results: Stool Consistency

- **Baseline**: Infant Stool Consistency (#d/total d)
  - Watery
  - Soft
  - Formed
  - Hard

- **Intervention**: Infant Stool Consistency (#d/total d)
  - Watery
  - Soft
  - Formed
  - Hard

- **Post-intervention**: Infant Stool Consistency (#d/total d)
  - Watery
  - Soft
  - Formed
  - Hard

Change in Infant Stool Consistency (%)

- **Intervention - Baseline**
  - Watery
  - Soft
  - Formed
  - Hard

- **Post-intervention - Intervention**
  - Watery
  - Soft

(*P<0.05)
Results: Infant Spit-ups and Flatulence

- Infant Spit-Ups (d/total d)
  - Baseline
  - Intervention
  - Post-intervention

- Infant Flatulence (d/total d)
  - Baseline
  - Intervention
  - Post-intervention
Results: Infant Tolerability

Infant Irritability

Baseline | Intervention | Post-intervention

Infant Upset

Baseline | Intervention | Post-intervention

Infant Discomfort

Baseline | Intervention | Post-intervention
## Results: Adverse Events

<table>
<thead>
<tr>
<th></th>
<th>BiLS (n = 34)</th>
<th>LS (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Intervention</td>
</tr>
<tr>
<td>Temperature Above 100.3F, (# d)</td>
<td>0.005</td>
<td>0.029</td>
</tr>
<tr>
<td>Blood in Stool, (# d)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Antibiotic Use, (# d)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Meds for Gas, (# d)</td>
<td>0.015</td>
<td>0.086</td>
</tr>
<tr>
<td>Jaundice diagnosis, % (n)</td>
<td>26.5% (9)</td>
<td>5.9% (2)</td>
</tr>
<tr>
<td>Colic, % (n)</td>
<td>0% (0)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Eczema diagnosis, % (n)</td>
<td>0% (0)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Illnesses, % (# reports)</td>
<td>2.9% (1)</td>
<td>11.8% (4)</td>
</tr>
<tr>
<td>Sick Doctor Visits, % (# reports)</td>
<td>2.9% (1)</td>
<td>15% (5)</td>
</tr>
</tbody>
</table>
Summary

• *B. infantis* EVC001 supplementation was found to be well-tolerated and did not result in a difference in the number or type of reported adverse events compared to non-supplemented infants.

• *B. infantis* EVC001 supplementation for 21 consecutive days mixed in breast milk resulted in fewer stools per day but more often softer than watery stools compared with non-supplemented infants possibly resembling a more mature gut than un-supplemented infants.
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QUESTIONS?

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