SYMBIOTA
SYNERGY IN MICROBIOTA RESEARCH

SHAPING THE GUT MICROBIOME DURING INFANCY

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UNIVERSITY OF ALBERTA
QUAECUMQUE VERA

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

ILSI NA Scientific Session, Bermuda, January 2018

From growing microbes to……

.....identifying them by their DNA

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@7_1_16901_1136
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+
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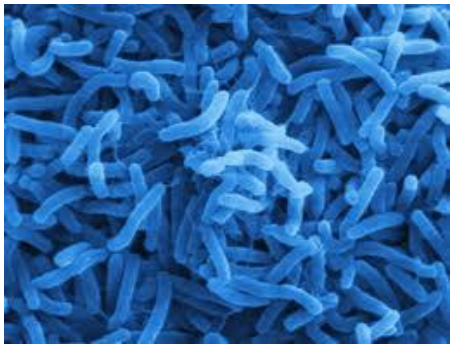
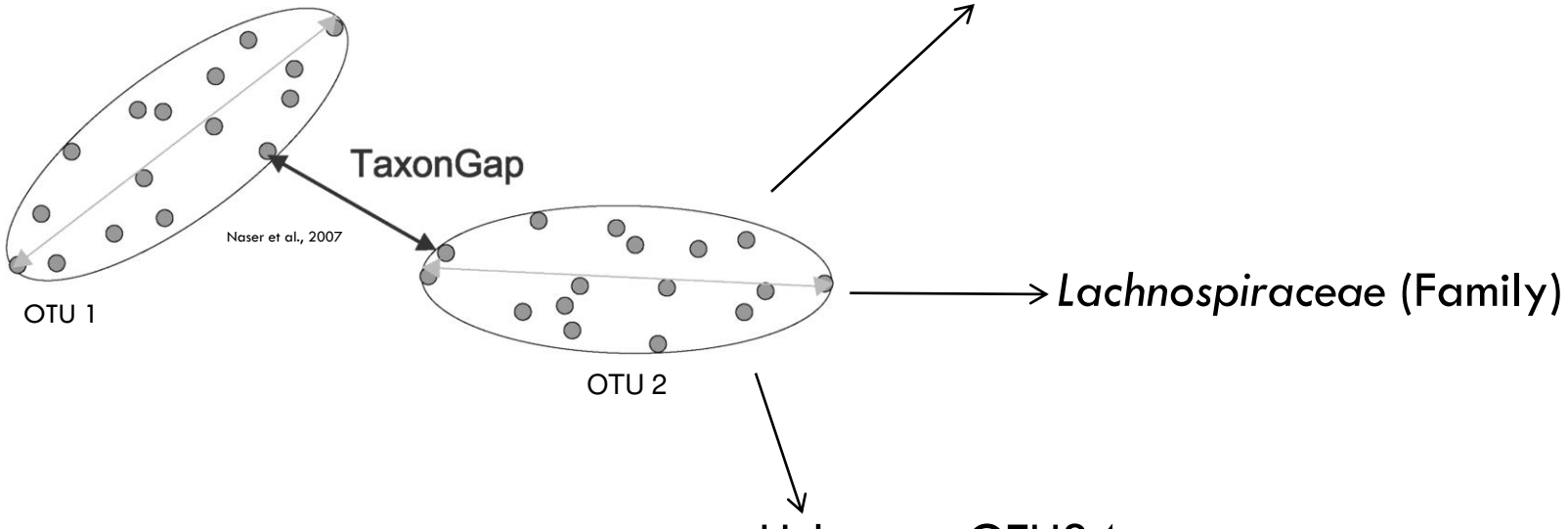
x80million

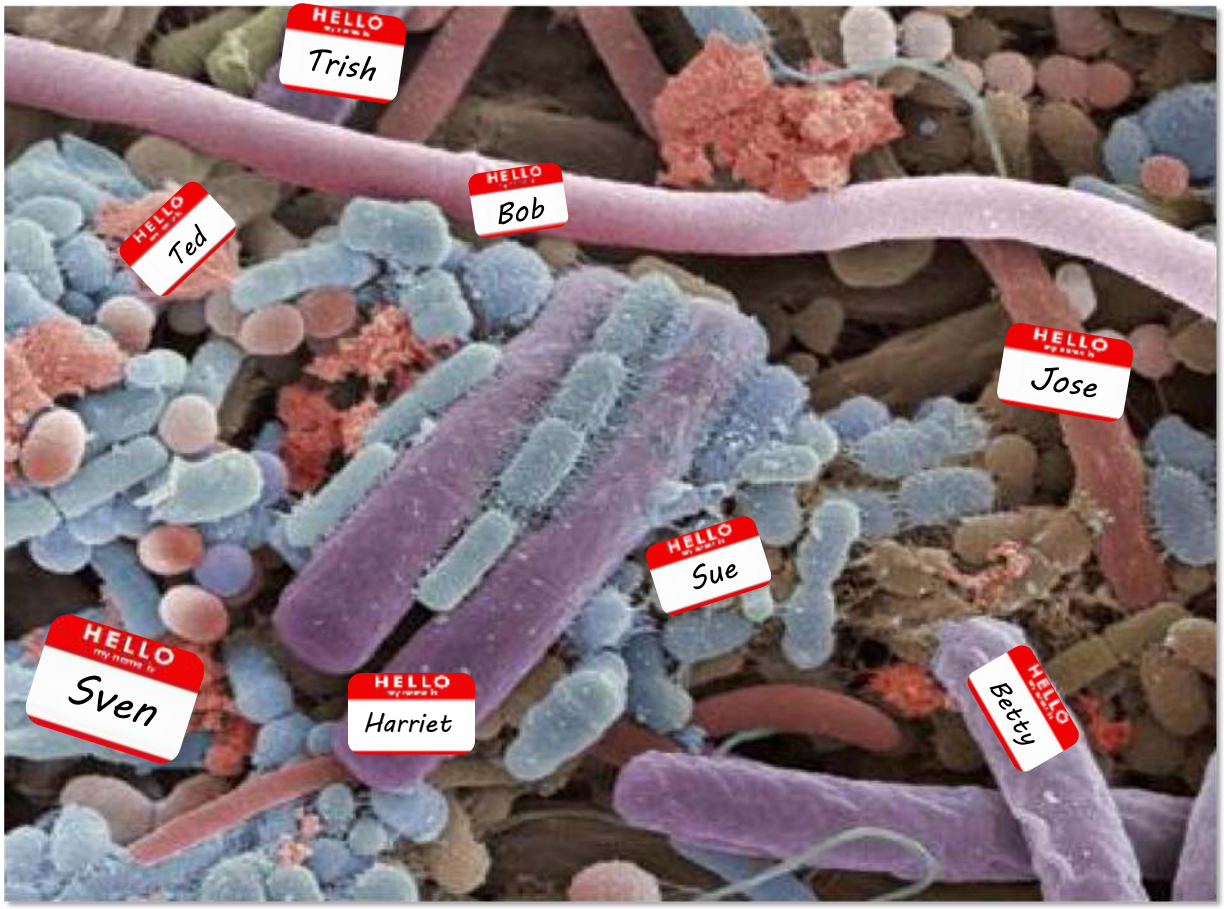
Streptococcus



Escherichia



More friends in the gut than you think



IMPACT OF EARLY LIFE EXPOSURES ON

by Mon Tun

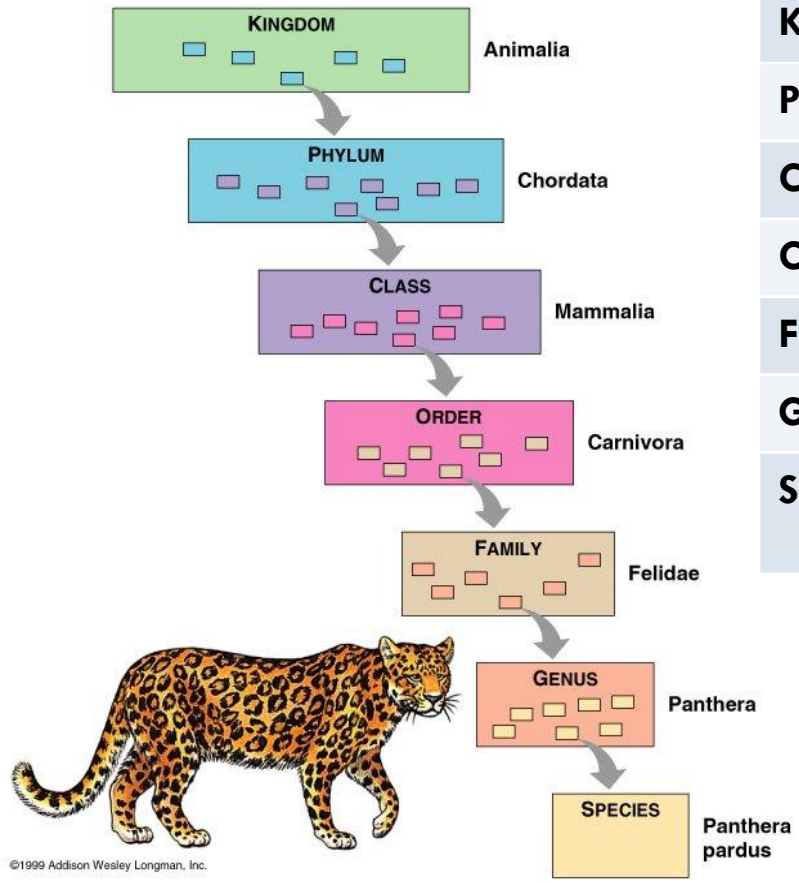
INFANT GUT MICROBIOTA

My session objectives are to:

- Introduce the SyMBIOTA (Synergy in Microbiota) research program & the CHILD (Canadian Healthy Infant Longitudinal Development) birth cohort

- Present findings on the maturation of the gut microbime over the 1st year of life and how this affected by early life exposures

Taxonomy 101 (Biological Classification)



	Human	*B. fragilis*	*C. difficile*
Kingdom	Animalia	Bacteria	Bacteria
Phylum	Chordata	Bacteroidetes	Firmicutes
Class	Mammalia	Bacteroidia	Clostridia
Order	Primates	Bacteroidales	Clostridiales
Family	Hominidae	Bacteroidaceae	Clostridiaceae
Genus	*Homo*	*Bacteroides*	*Clostridium*
Species	*Homo sapiens*	*Bacteroides fragilis*	*Clostridium difficile*

The Canadian Healthy Infant Longitudinal Development (CHILD) Study

How do **genes** and the **environment** influence child health and development?

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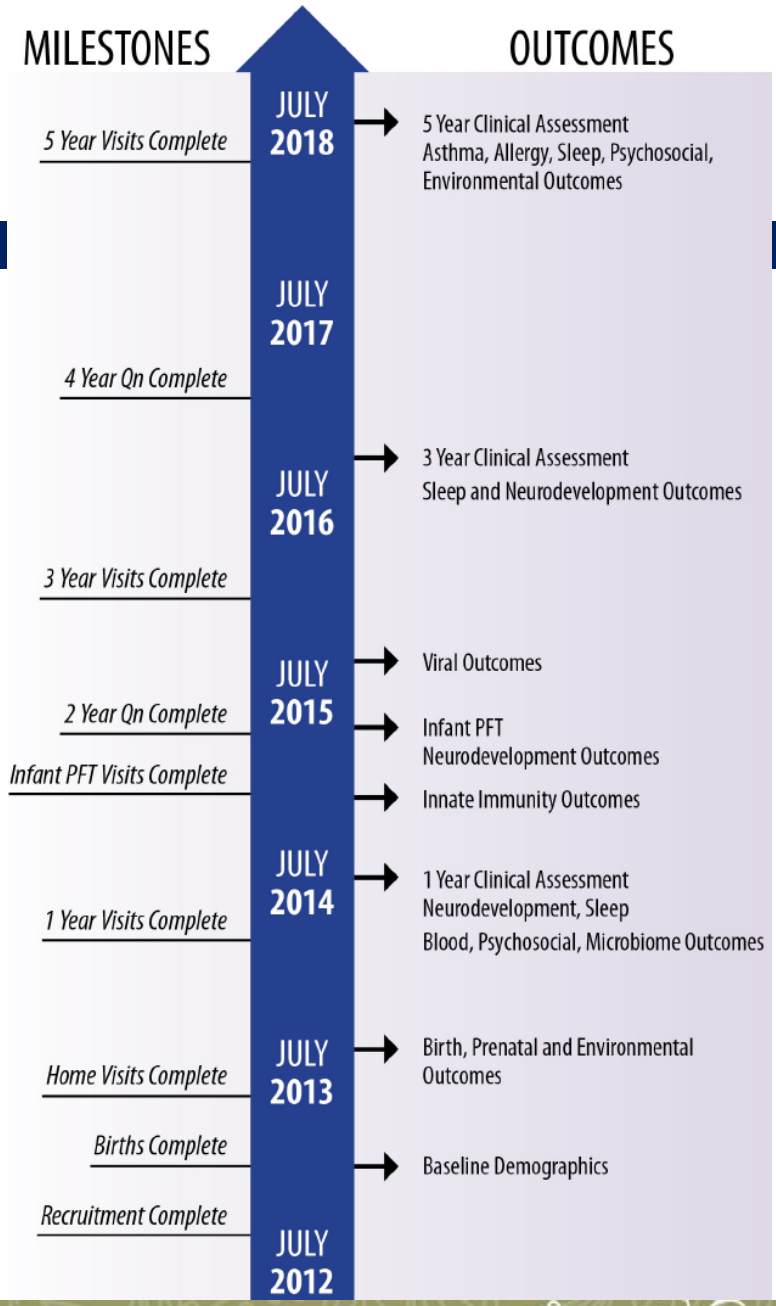
AllerGen Allergy, Genes and Environment Network / Le réseau des allergies, des gènes et de l'environnement

CHILD Study

HELP CHILDREN GROW UP HEALTHY

$30M	Invested
500,000	Biological Samples Banked
200,000	Questionnaires Completed
3600	Families Participating
92%	Retention at 1 year
40+	Senior Researchers
20+	Scientific Disciplines:

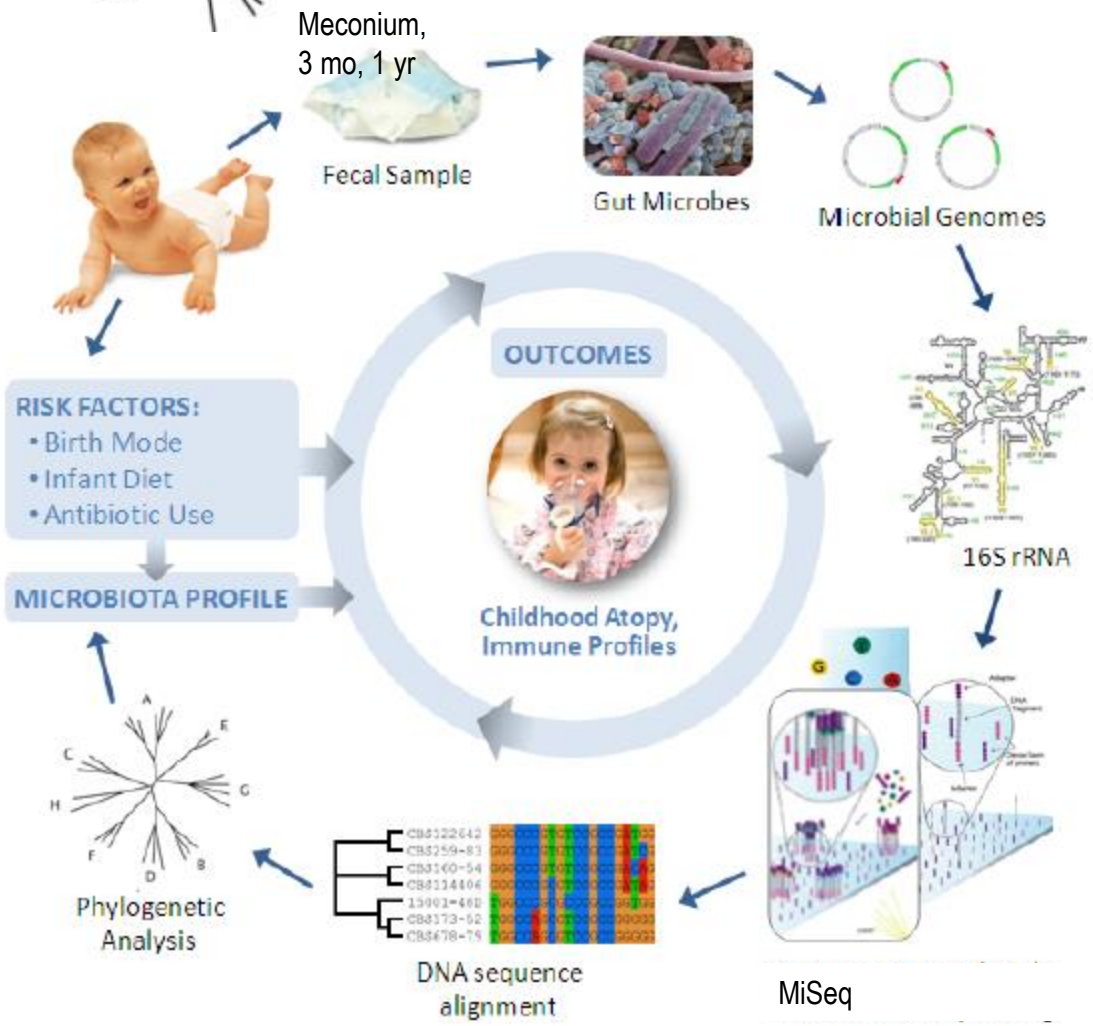
Air Quality	Infectious Disease	Physiology
Biostatistics	Molecular Biology	Population Health
Endocrinology	Neonatology	Psychology
Environmental Health	Neuroimmunology	Respirology
Epidemiology	Nutrition	Sociology
Genetics	Obstetrics	Toxicology
Immunology	Pediatrics	**Microbiome**



SyMBIOTA

SYNERGY IN MICROBIOTA RESEARCH

www.allergen-nce.ca/Research/SyMBIOTA.html



Metadata and infant fecal samples from the Canadian Healthy Infant Longitudinal cohort (www.childstudy.ca)

CHILD Study
HELP CHILDREN GROW UP HEALTHY

AllerGen

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

CIHR CDN MICROBIOME INITIATIVE TEAM GRANT, $2.5 million/5 yr

GUT MICROBIOME MATURATION IN FULL TERM INFANTS

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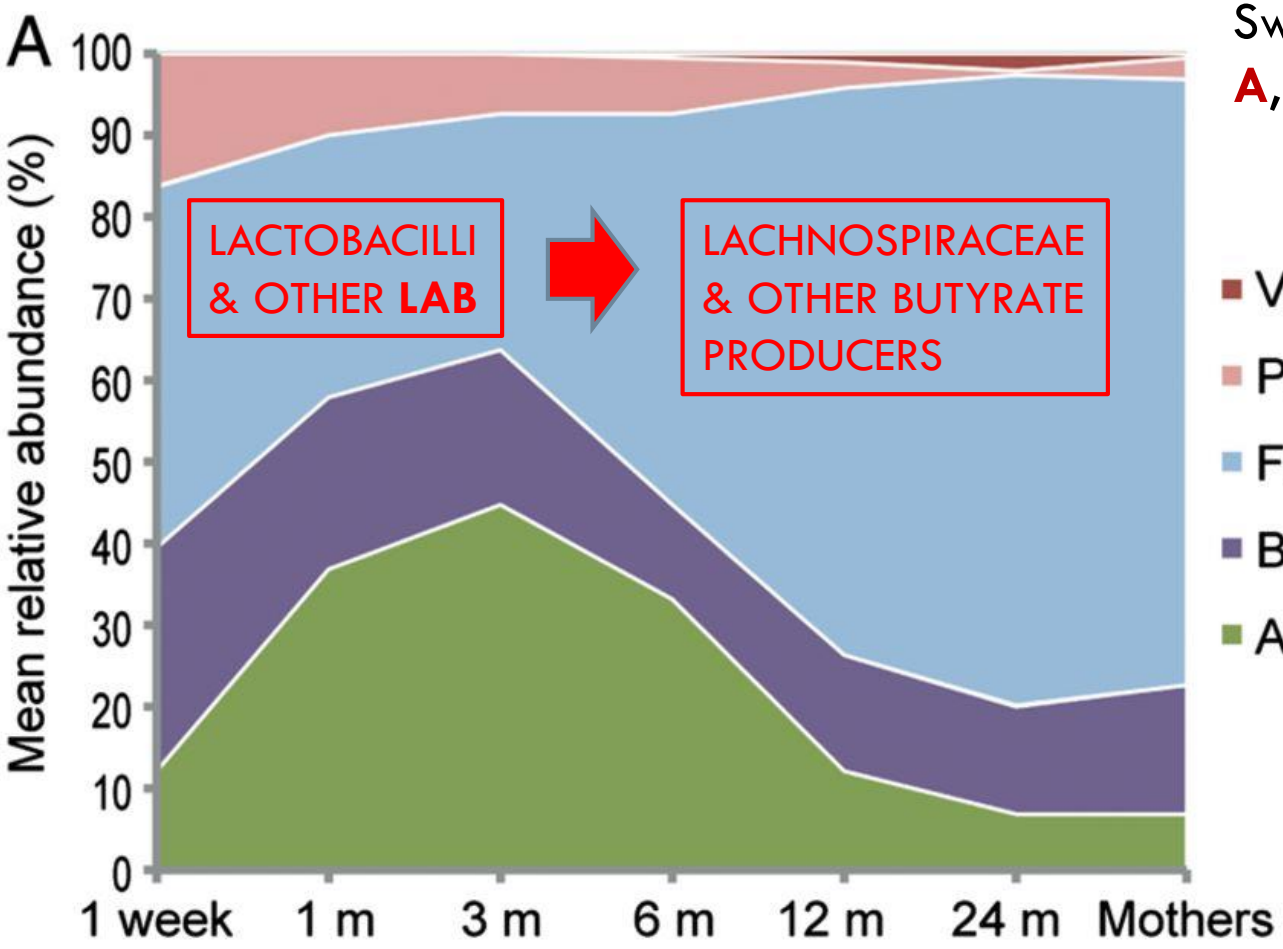
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

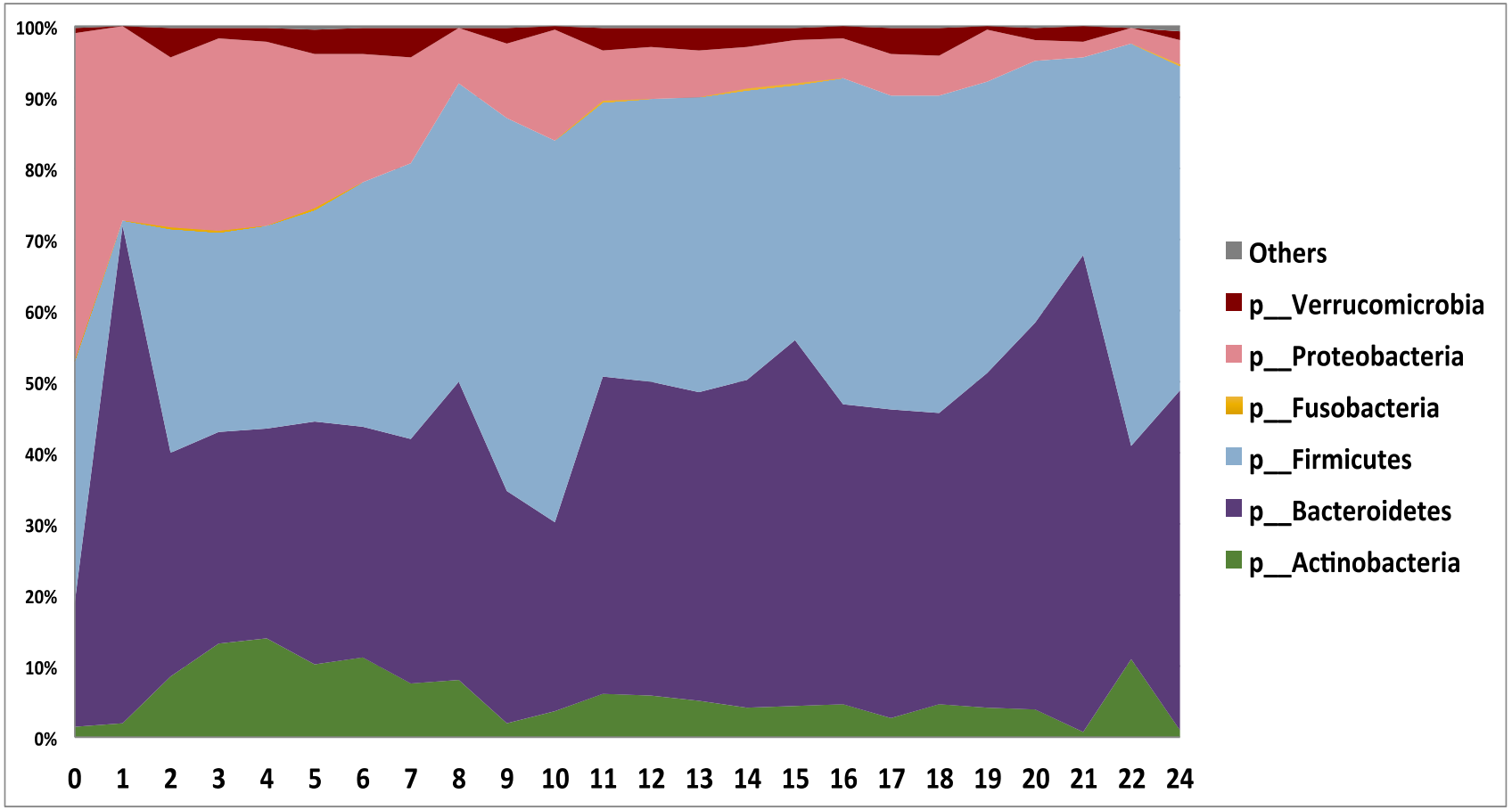
Development of gut microbiota over the first years of life (Jakobsson al. Gut 2014; 63)



Swedish birth cohort

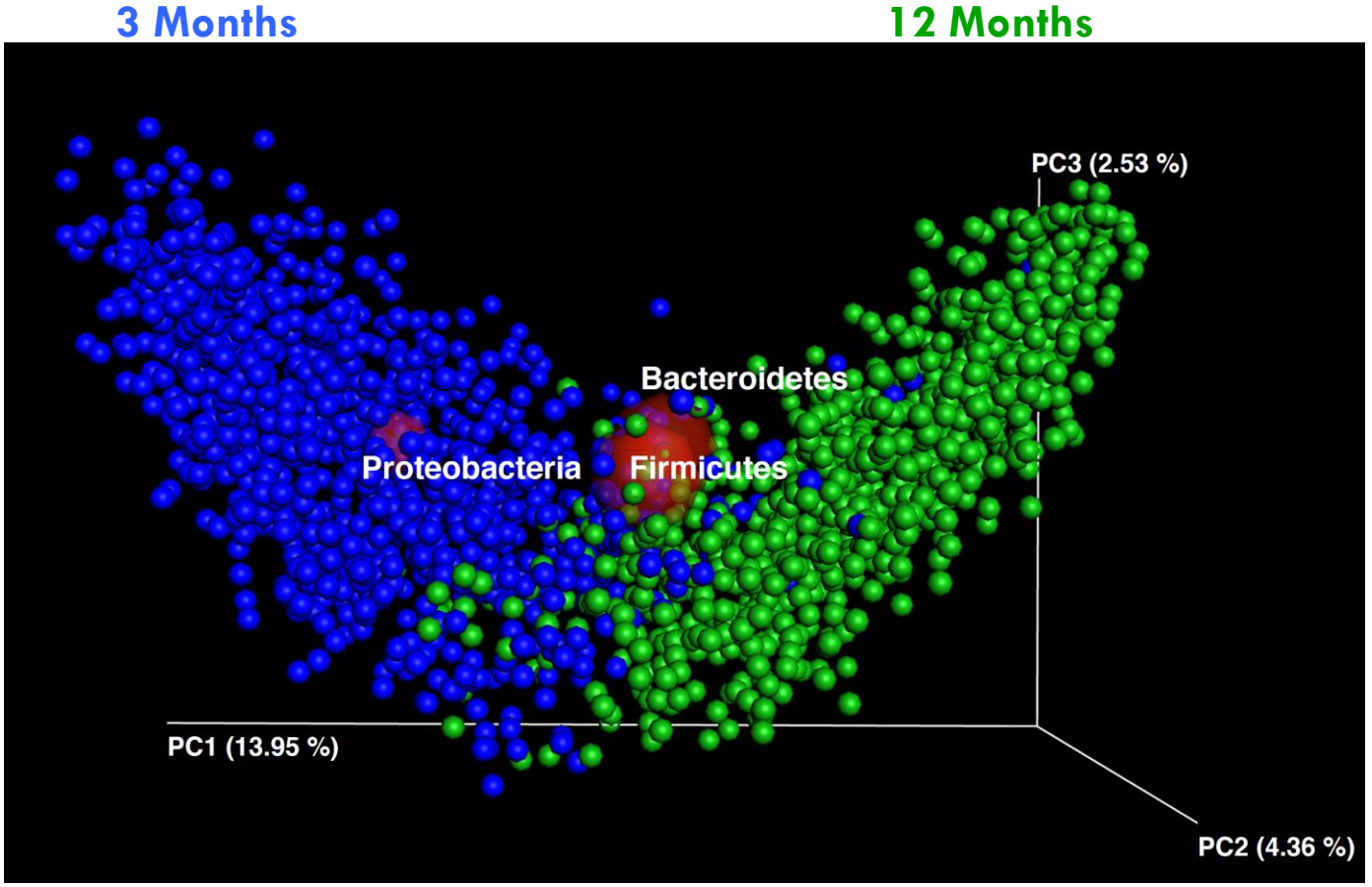
A, vaginally born infants

Mean relative abundance (%) of microbial phyla



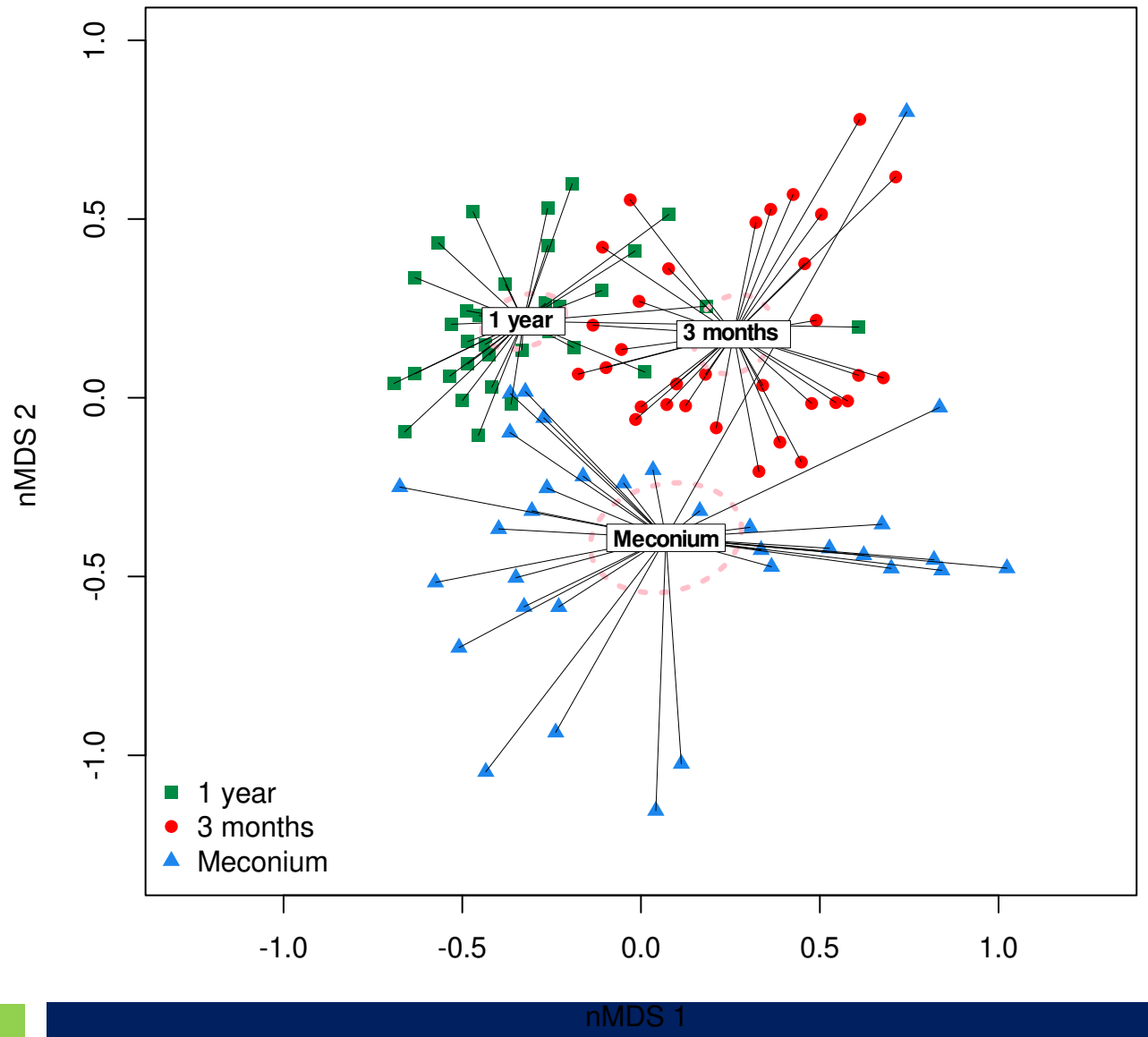
CHILD COHORT

Development of gut microbiome during infancy



CHILD COHORT

Development of gut microbiome during infancy



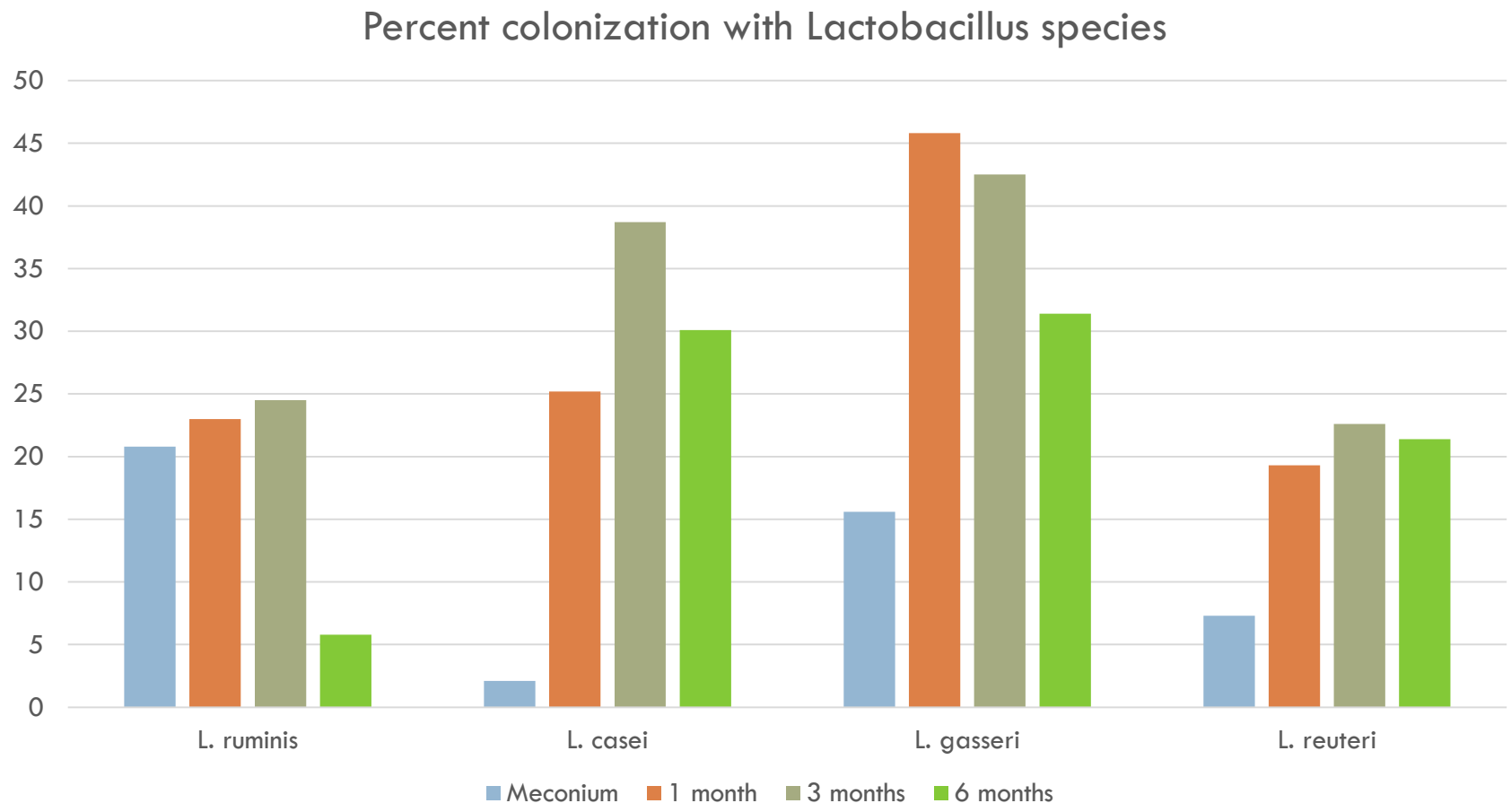
CHILD COHORT

Development of gut microbiome during infancy

Important Role of Pioneer Gut Microbes

- Days after birth, facultative anaerobes, *LAB (lactic acid bacteria, eg. lactobacilli, streptococci) and Enterobacteriaceae*
  - create an anaerobic environment allowing strict anaerobes, such as bifidobacteria, *Clostridium* and *Bacteroides* species, to thrive
- Early microbial composition determines future composition
  - Heavier colonization with *Enterobacteriaceae* within 3 days of birth = greater abundance of bifidobacteria 6 months later (Dogra et al. 2015)

Lactobacilli (of Firmicutes) decline in gut microbiota with advancing infant age



Martin et al. Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLOS One 2016; 11

How you are born, what you are fed and your exposure to antibiotics shapes your microbiome as an infant !!!!!!

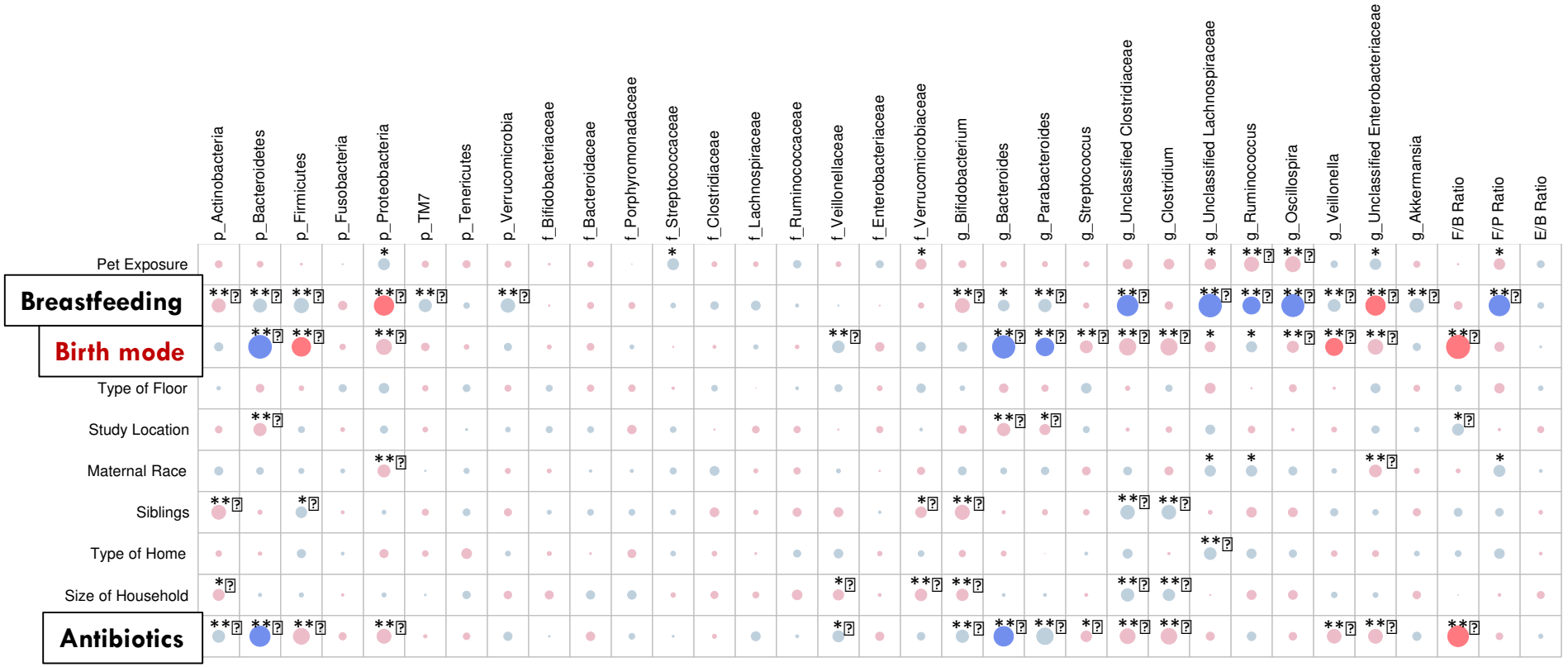


Figure is available in: Tun et al. Exposure to household furry pets influences the gut microbiota of infant at 3-4 months following various birth scenarios Microbiome (2017) 5:40

CESAREAN BIRTH & GUT DYSBIOSIS IN EARLY INFANCY

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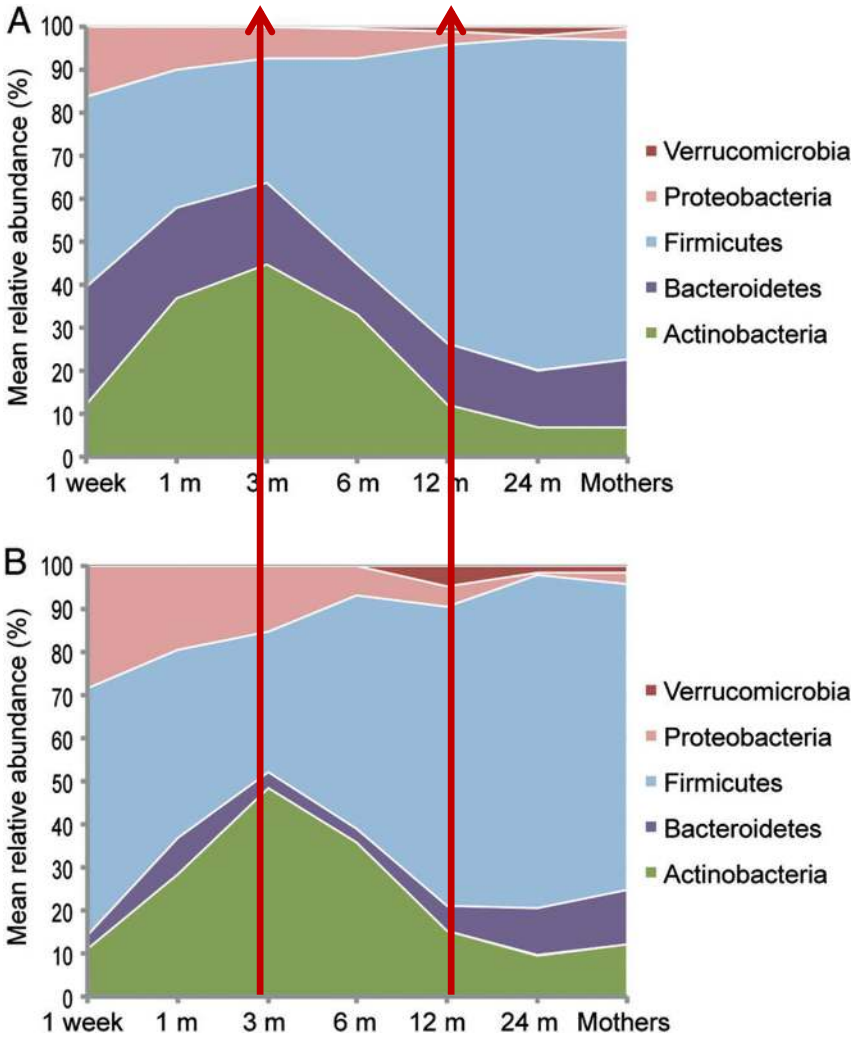
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Development of infant gut microbiota over the first years of life (Jakobsson al. Gut 2014; 63)



Swedish birth cohort

A, vaginally born infants

B, cesarean born infants

DELAYED Bacteroides colonization

Breastfeeding and a Vaginal Birth Healthier, Study Says

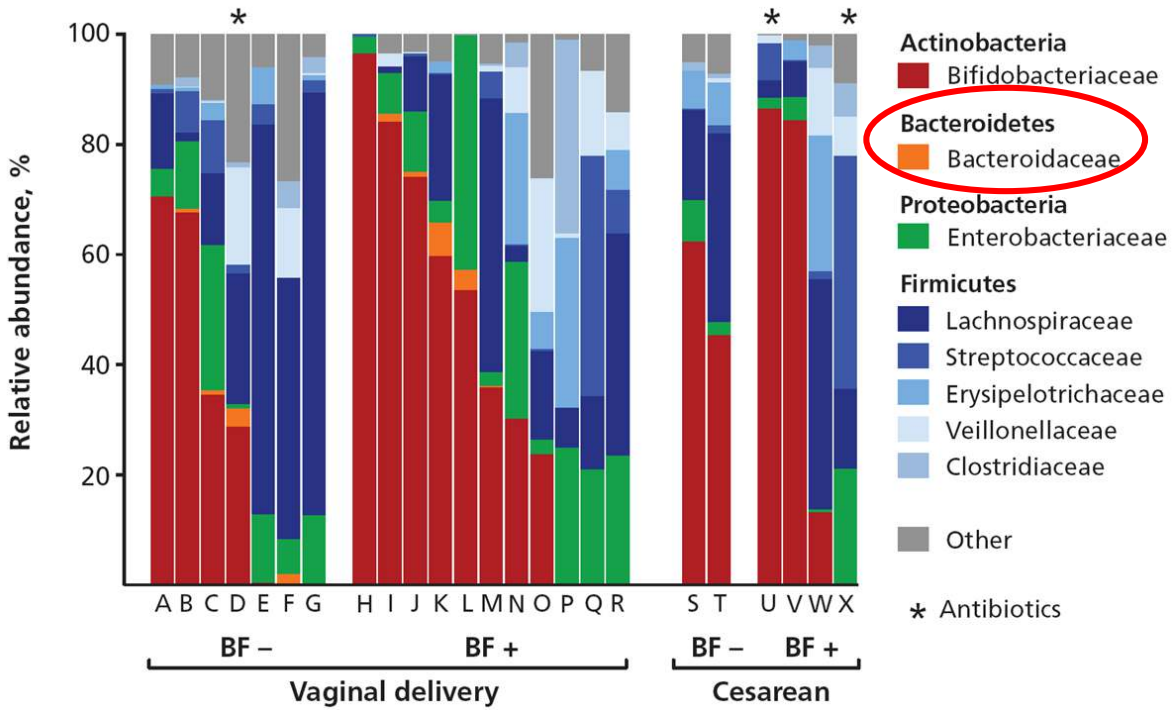
By: Kylie McConville

Photo: Thinkstock / The Bump

CMAJ RESEARCH

Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months

Meghan B. Azad PhD, Theodore Konya MPH, Heather Maughan PhD, David S. Guttman PhD, Catherine J. Field PhD, Radha S. Chari MD, Malcolm R. Sears MB, Allan B. Becker MD, James A. Scott PhD, Anita L. Kozyrskyj PhD, on behalf of the CHILD Study Investigators



Researchers studied 24 babies and compared the bacteria found in baby poop samples collected when each infant was just 3 months old. They knew, prior to the study, that C-section deliveries could result in a higher risk of asthma, diabetes, cancer and even obesity, but they didn't know *how*. Their recent work suggests that at least part of that risk may be due to the microbes forming inside baby.

Contributing emerging microbiome evidence to clinical practice

- Kozyrskyj AL, Bridgman SL, Tun MH. Impact of Pre and Postnatal Medical Interventions on Infant Gut Microbiota in: **Microbiota in Health and Disease: From Pregnancy to Childhood**, Browne P, Claassen E, Cabana M (eds). Wageningen Academic Publisher, 2017. **THIS CHAPTER IS OPEN ACCESS**

- Kozyrskyj AL, Tun HM, Bridgman SL. The Microbiome and Control of Weight Gain in: **Pediatric Obesity: Etiology, Pathogenesis & Treatment**, Freemark M (ed). Springer Publisher, USA, 2018

Chapter 4: Birth & Postnatal Interventions

- Chapter 4 is organized by 4 themes:
  - Birth mode (vaginal birth vs scheduled or emergency cesarean)
  - Intrapartum maternal antibiotic prophylaxis (IAP for GBS)
  - Extended hospitalization post birth
  - Postnatal infant IV and oral antibiotic treatment

- Chapter table organized by the same themes plus
  - Taxon category of major changes ie. *Enterobacteriaceae, Bacteriodaceae,*

Chapter 4: TAKE HOME MESSAGE

- All 4 main early life exposures during infancy
  - ↓ Bacteroidetes species (see Rutayisire E et al systematic review), required for the maintenance of the gut mucin barrier
  - ↓ bifidobacteria
  - ↑ staphylococci
  - ↑ Clostridium (difficile)
  - ↑ Proteobacterial species
- Emergency cesarean and infant antibiotic use
  - ↑ enterococci

IAP, CESAREAN BIRTH & INFANT GUT DYSBIOSIS AT 3 MONTHS OF AGE

Azad et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG 2016;123(6):983-93

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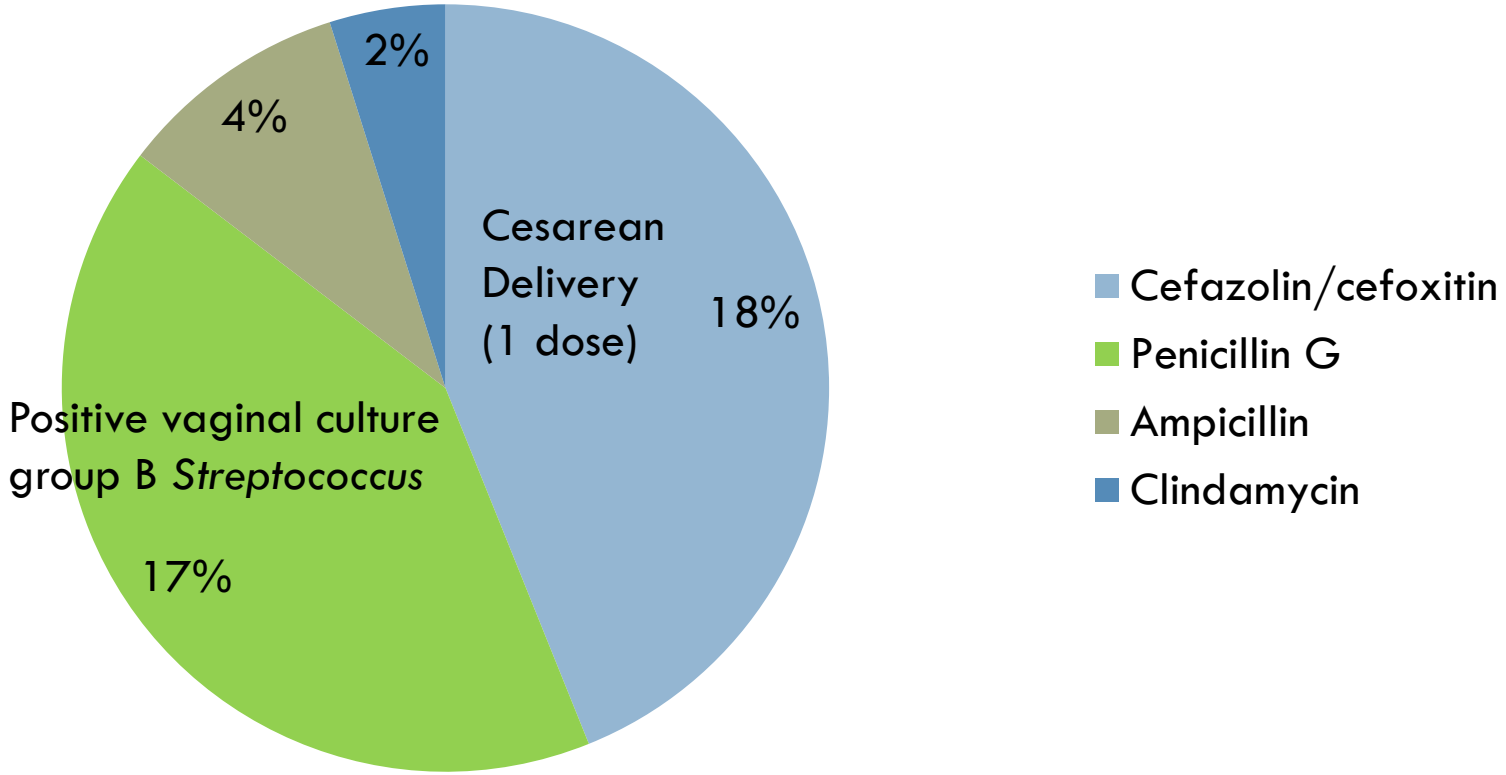
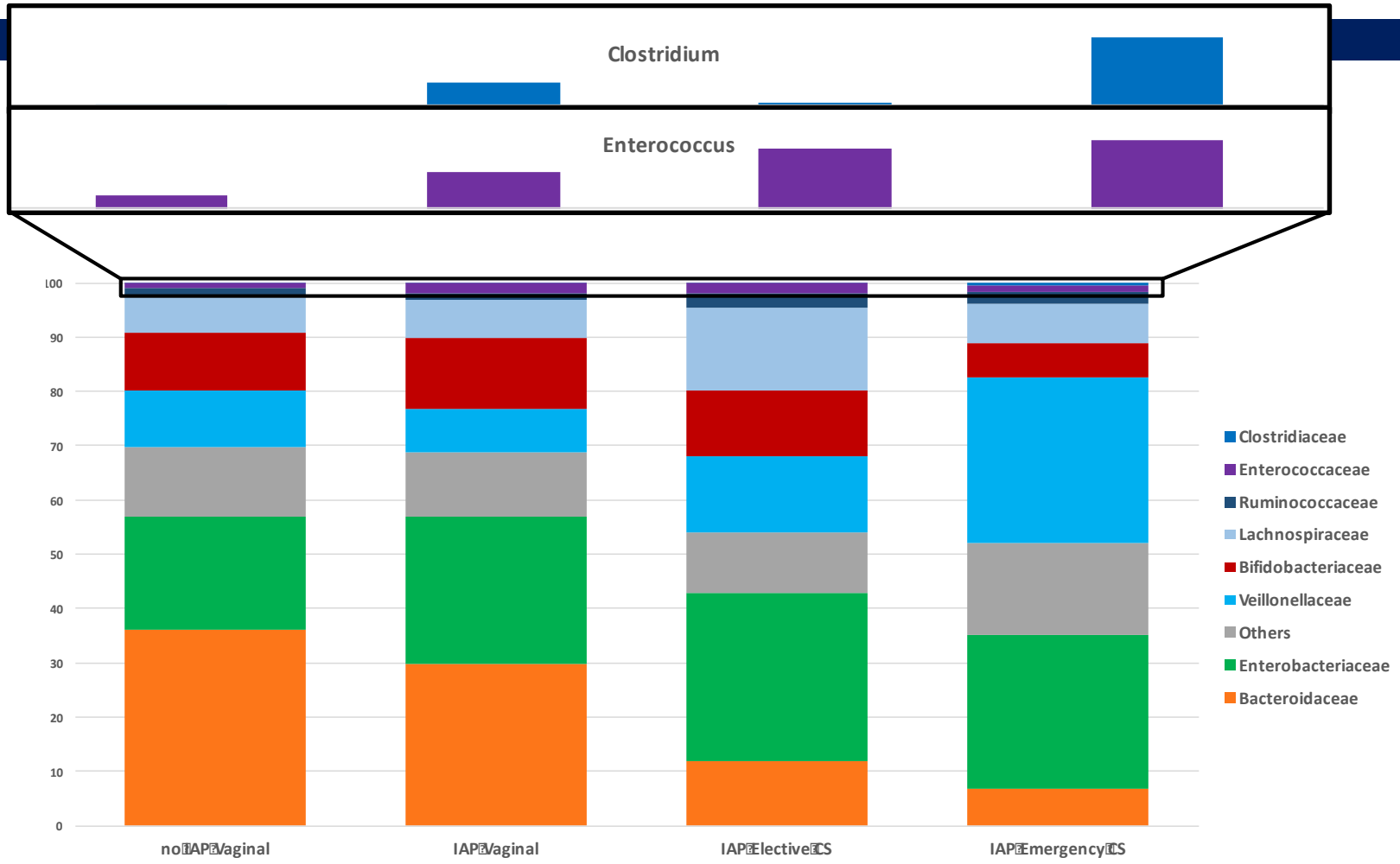
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Almost 40% of infants are exposed to an antibiotic by the time they are born



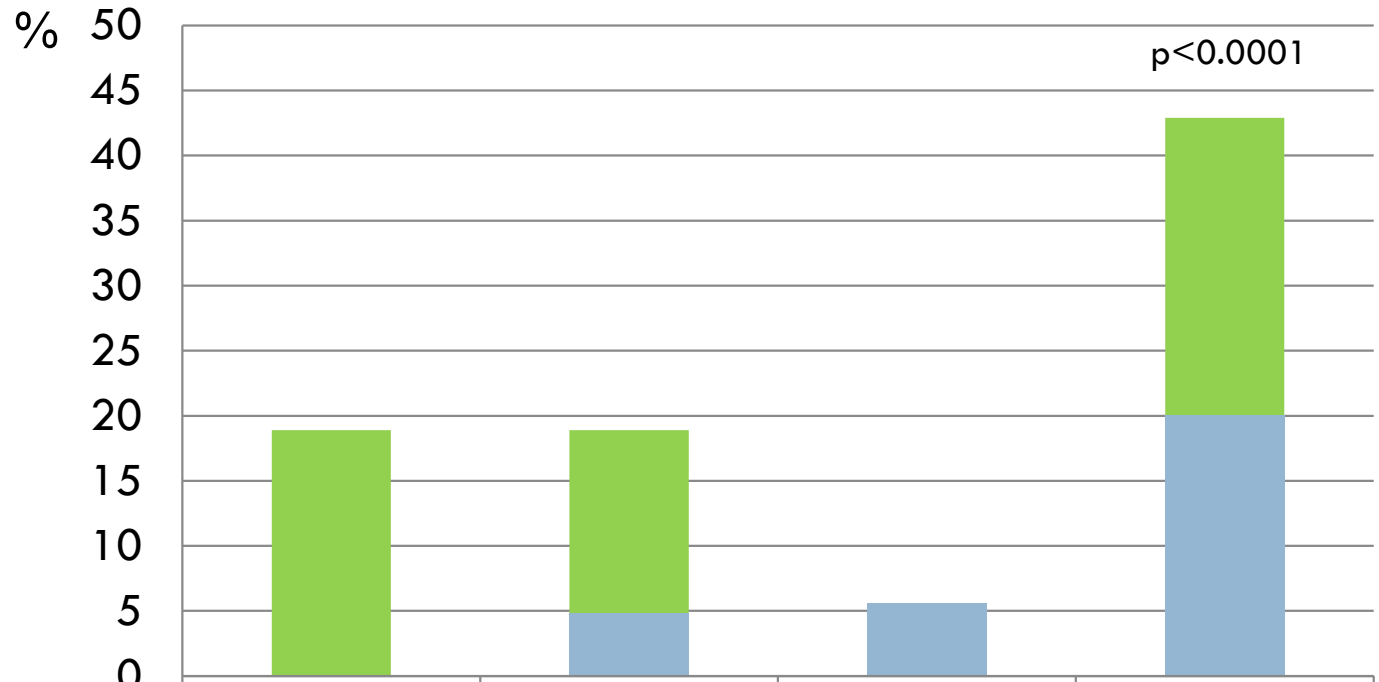
Persaud RR et al. Perinatal antibiotic exposure of neonates in Canada and associated risk factors: a population-based study. J Matern Fetal Neonat Med 2014: 1-6.

Bacteroidaceae abundance (orange) is reduced with IAP and CS. Other ↑s typical of antibiotic resistance.



Azad et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG 2016;123

The special case of emergency CS; mothers and newborns were more likely to receive antibiotics



	no IAP, vaginal	IAP, vaginal	IAP, elect CS	IAP, emerg CS
Maternal PP antibiotics	18.9	18.9		42.9
Newborn antibiotics	0	4.8	5.6	20

HOSPITALIZATION POST BIRTH & GUT DYSBIOSIS AT 3-4 MONTHS OF AGE

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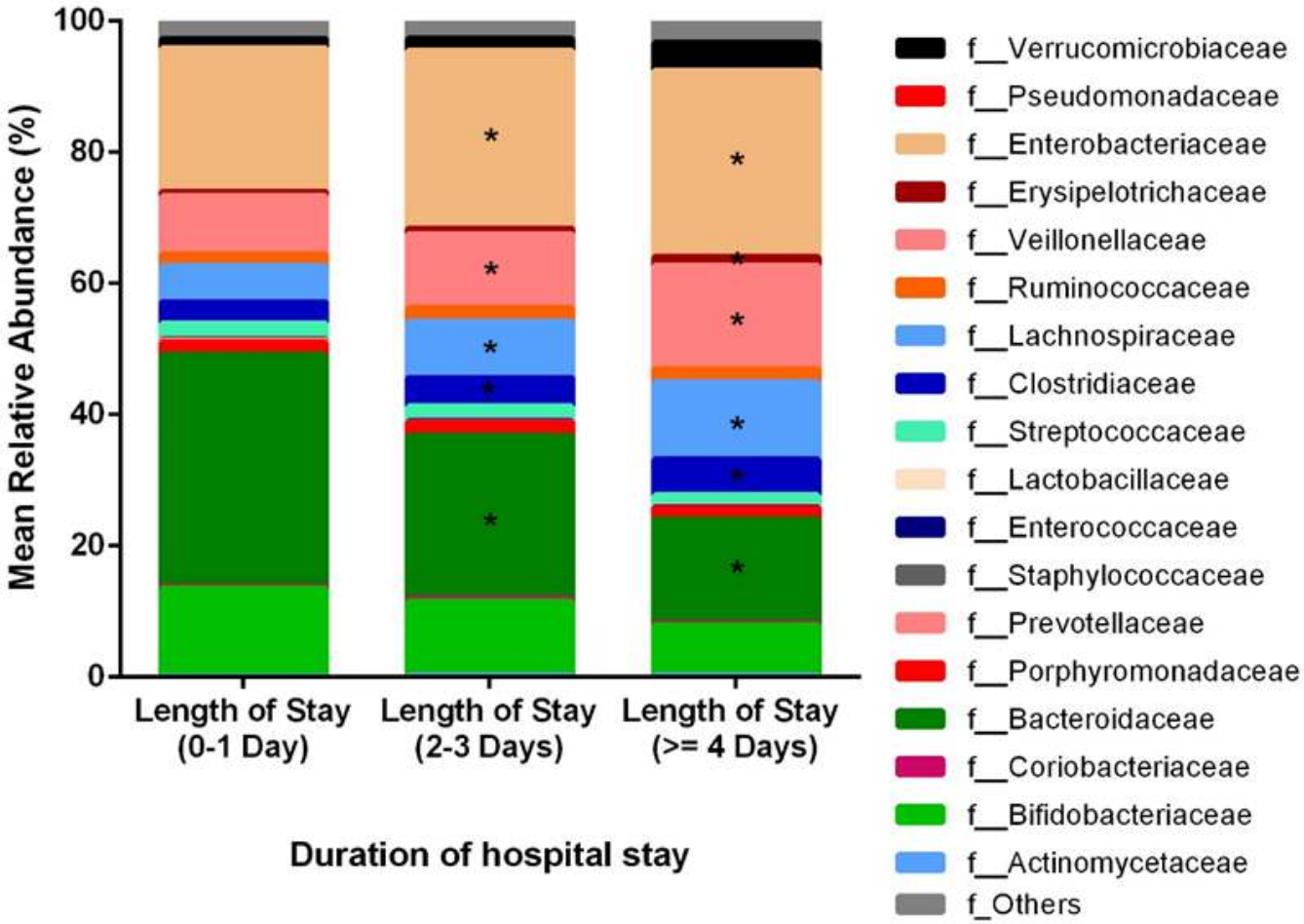
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Prolonged hospital stay after birth: ↓Bacteroidaceae but ↑Lachnospiracaea, Enterobacteriaceae



DEVIATION IN GUT MICROBIAL DEVELOPMENT IN LATER INFANCY WHEN NOT BORN VAGINALLY OR BREASTFED

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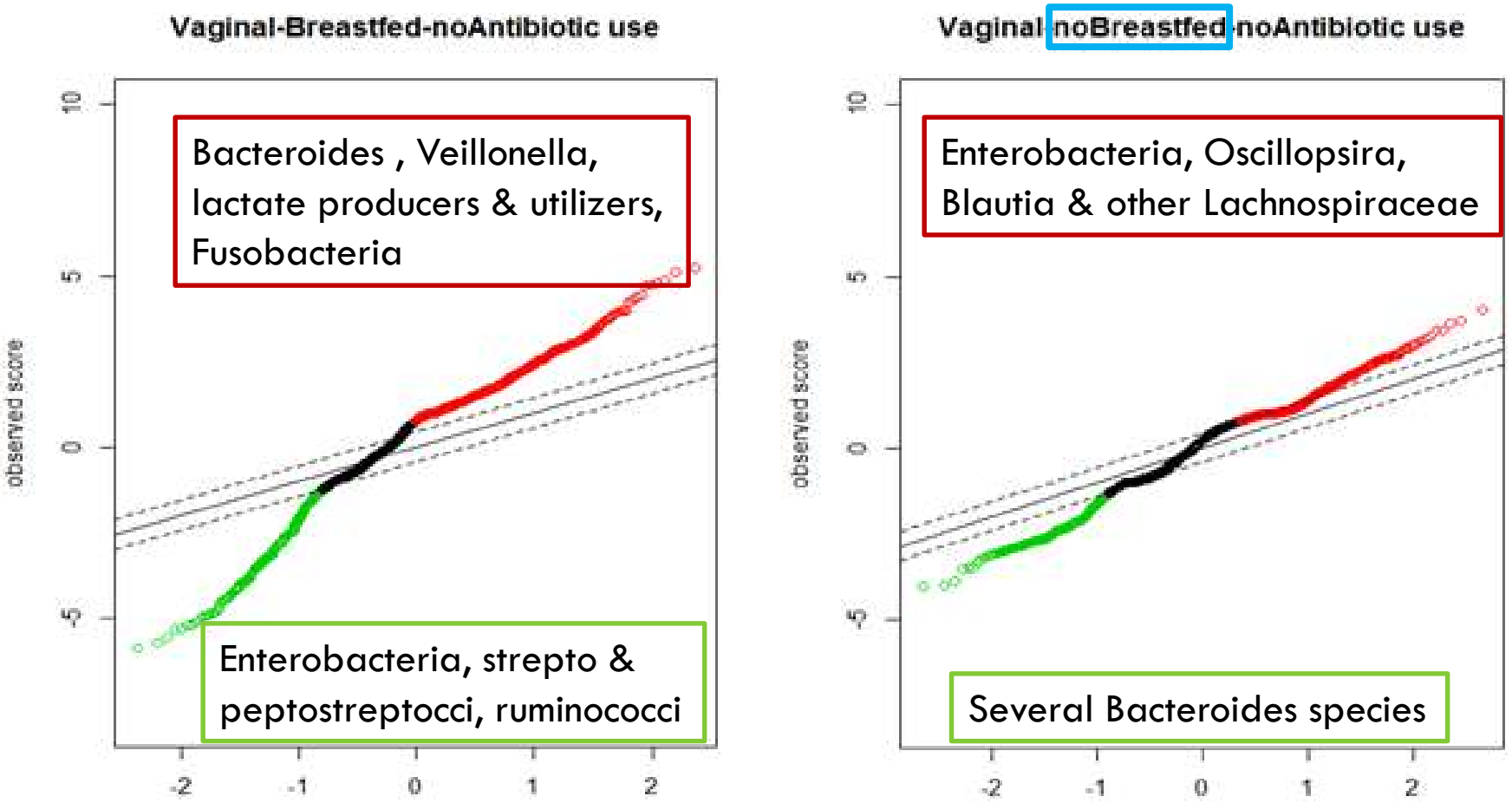
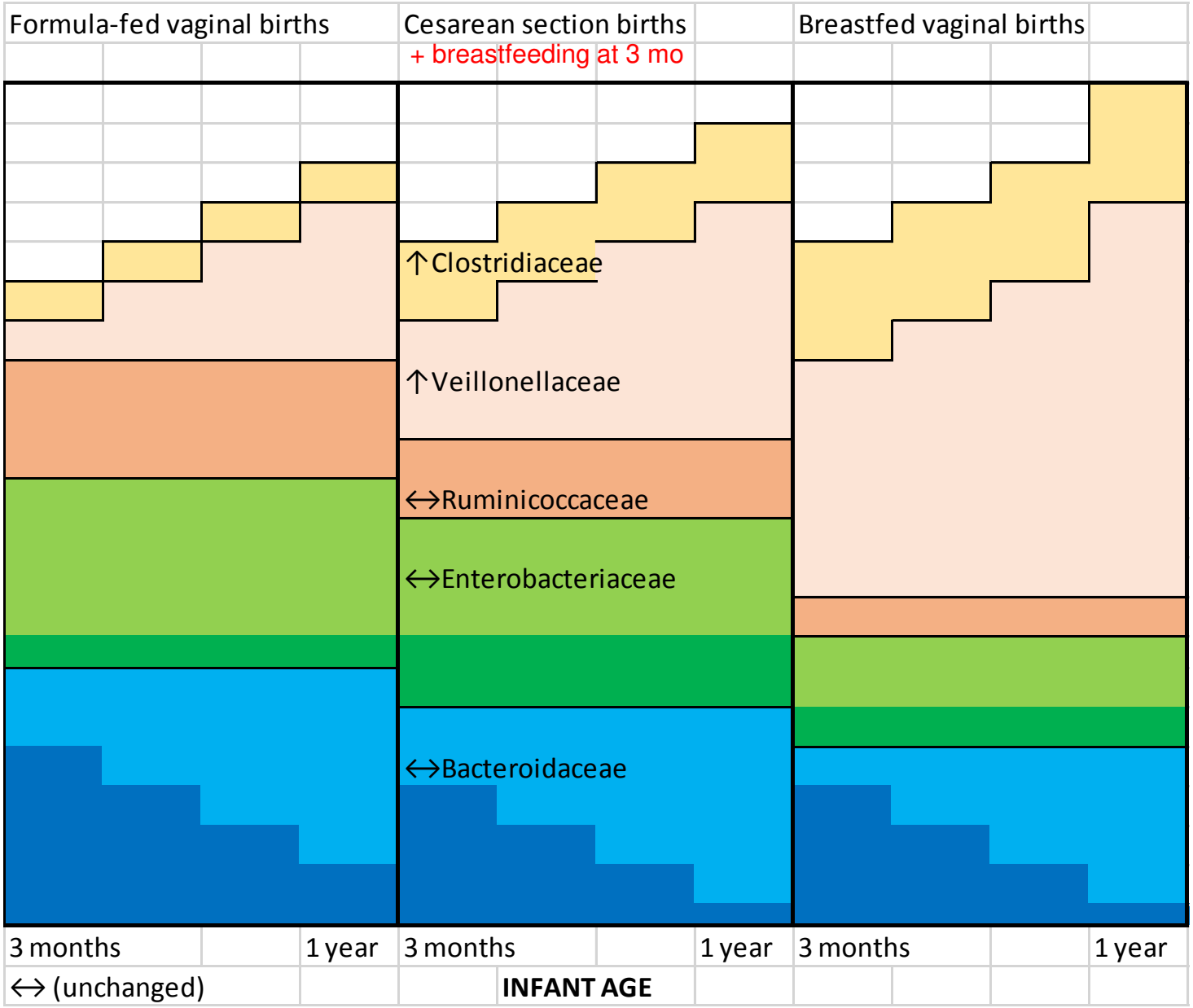
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Few taxon ↓ or ↑ between 3 months -1 year of age in infants not breastfed = higher risk for food sensitization



Yasmin et al. Cesarean Section, Formula Feeding, and Infant Antibiotic Exposure: Separate and Combined Impacts on Gut Microbial Changes in Later Infancy. Frontiers Pediatr 2017



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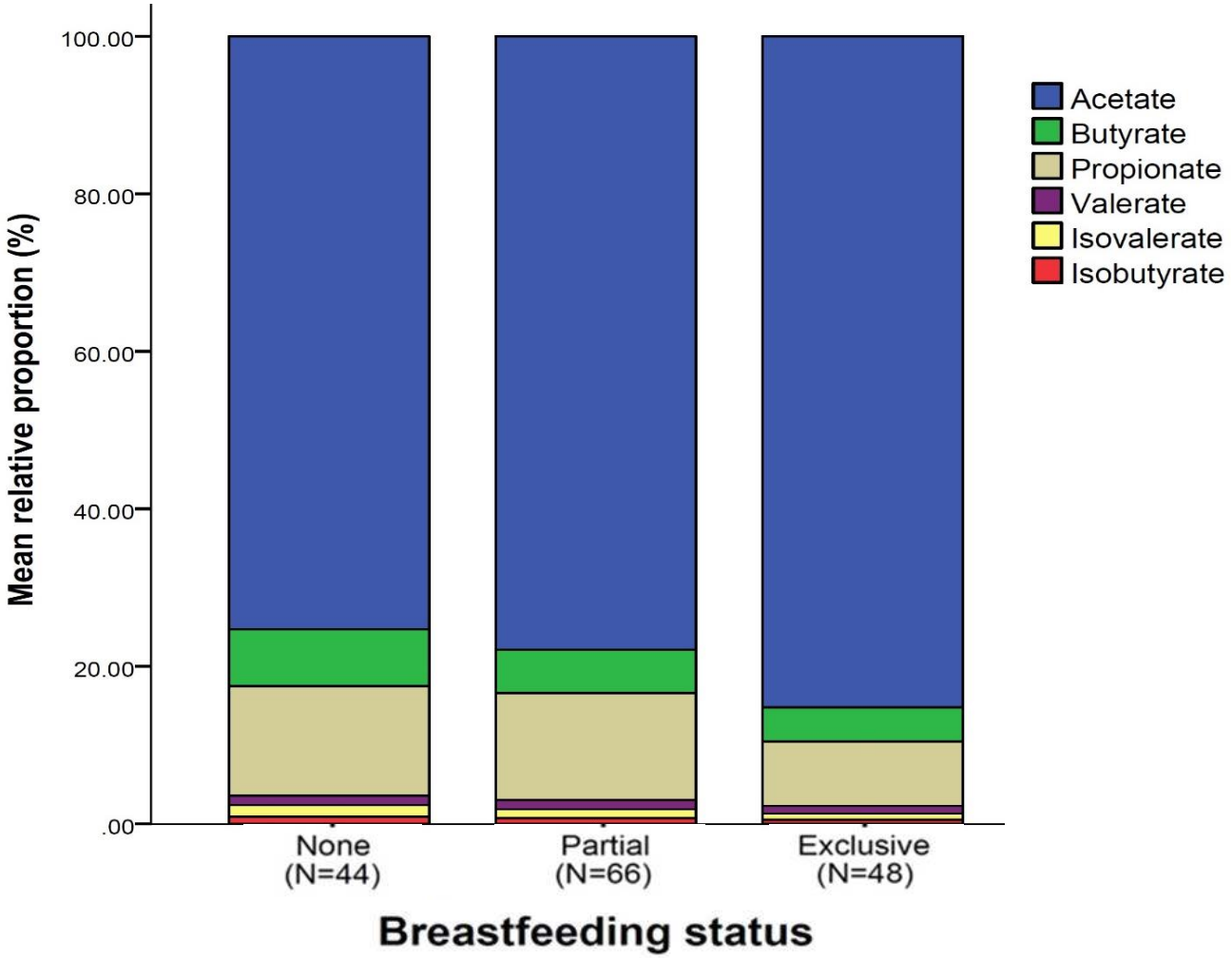
Yasmin et al. Cesarean Section, Formula Feeding, and Infant Antibiotic Exposure: Separate and Combined Impacts on Gut Microbial Changes in Later Infancy. Frontiers Pediatr 2017

MODERATING EFFECT OF EARLY BREAST FEEDING IN CESAREAN DELIVERY

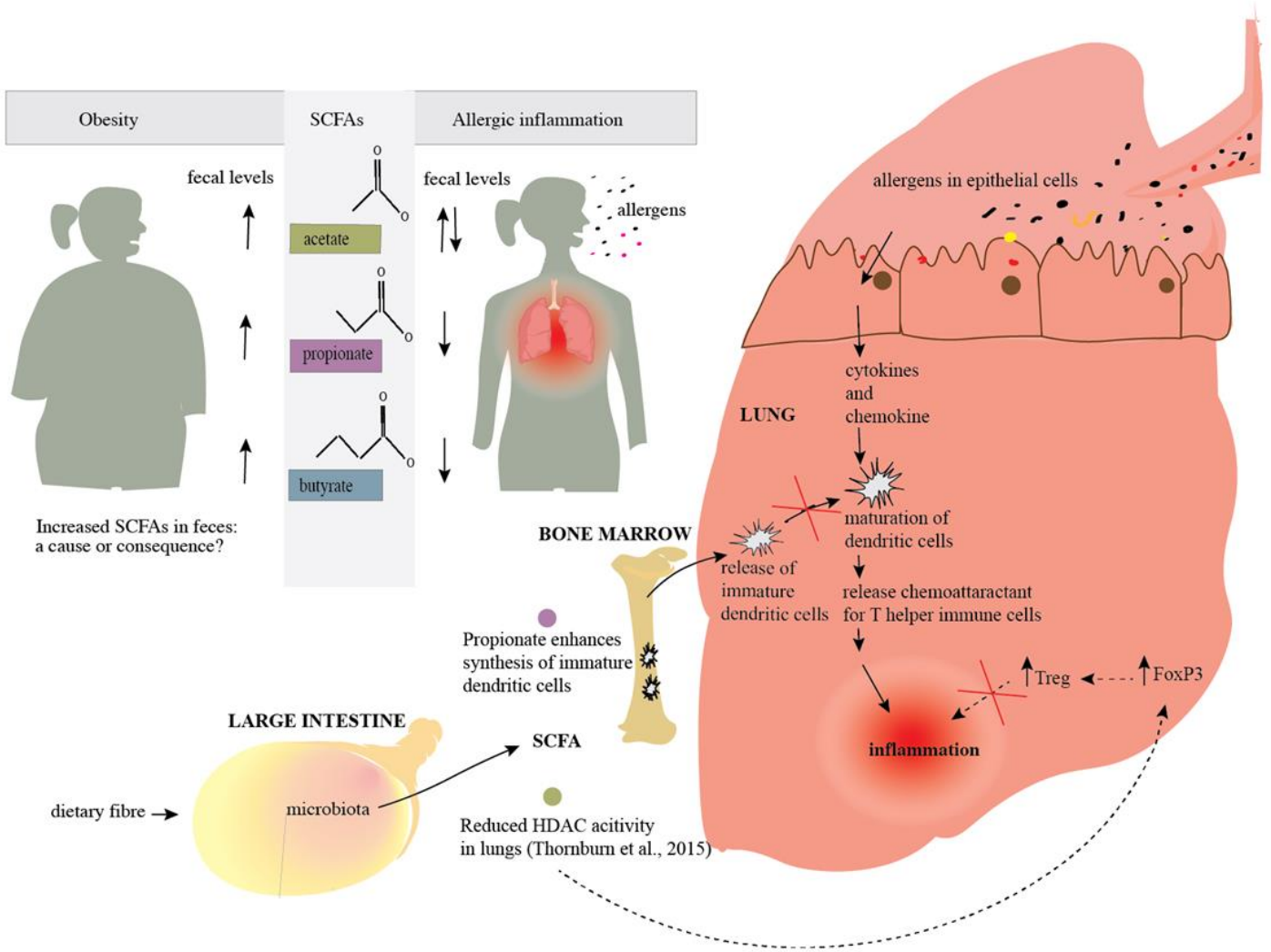
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Short-chain fatty acid (SCFA) produced by gut microbes in infants breastfed vs not: Total SCFA ↓ … **BUT ↑ % acetate** (Bridgman et al. Frontiers Nutr 2017;4)

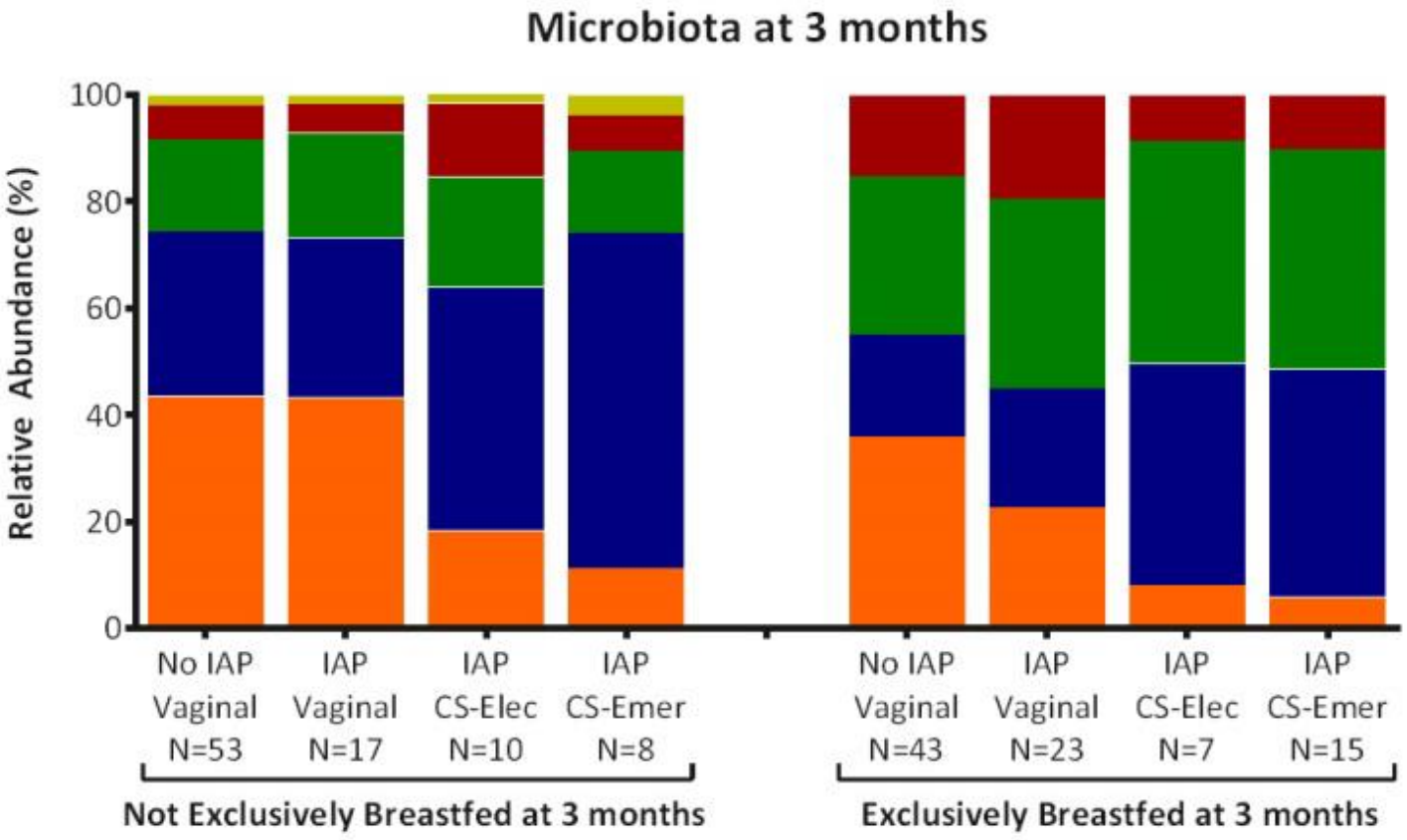


SCFA, acetate and propionate, reduce risk for allergic asthma in animal models

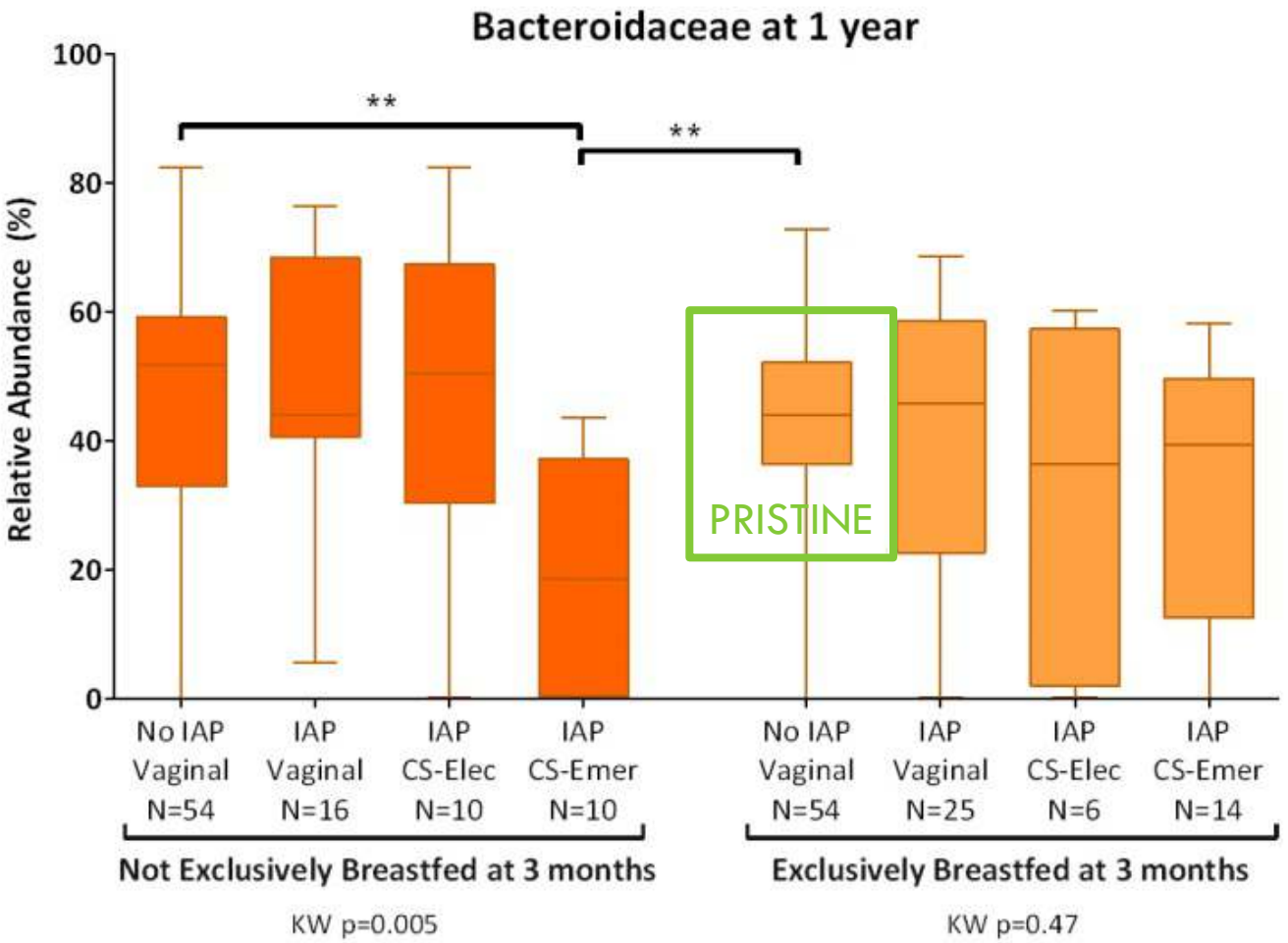
SCFA metabolites produced by gut microbes affect leptin & other satiety hormones, gluconeogenesis and lipid storage: Kumari & Kozyrskyj. Obes Rev 2017; 18



Cesarean section leaves an imprint (↓ Bacteroidetes in orange) at 3 months independent of breastfeeding

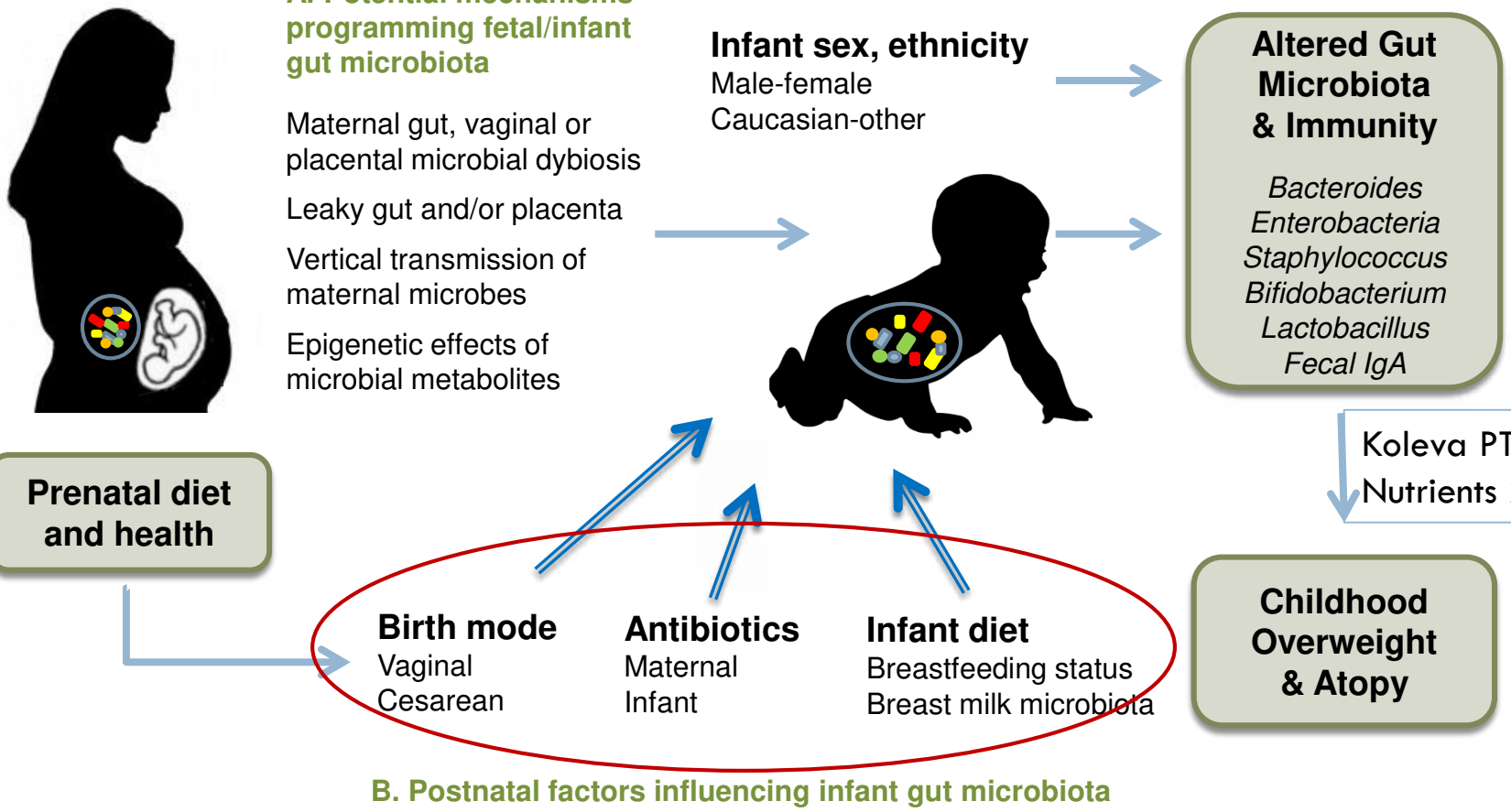


But in the absence of exclusive breastfeeding at 3 months ↓ Bacteroidaceae persists after emergency CS



AND Pregnancy matters……

Kozyrskyj AL et al. Fetal programming of overweight through the microbiome: boys are disproportionately affected. J Dev Orig Health Dis 2016; 7: 25-34



Koleva PT et al. Microbial programming of health and disease starts during fetal life. Birth Defects Res 2015; 105:265–277

PRENATAL INFLUENCE OF MATERNAL OVERWEIGHT, STRESS & ASTHMA

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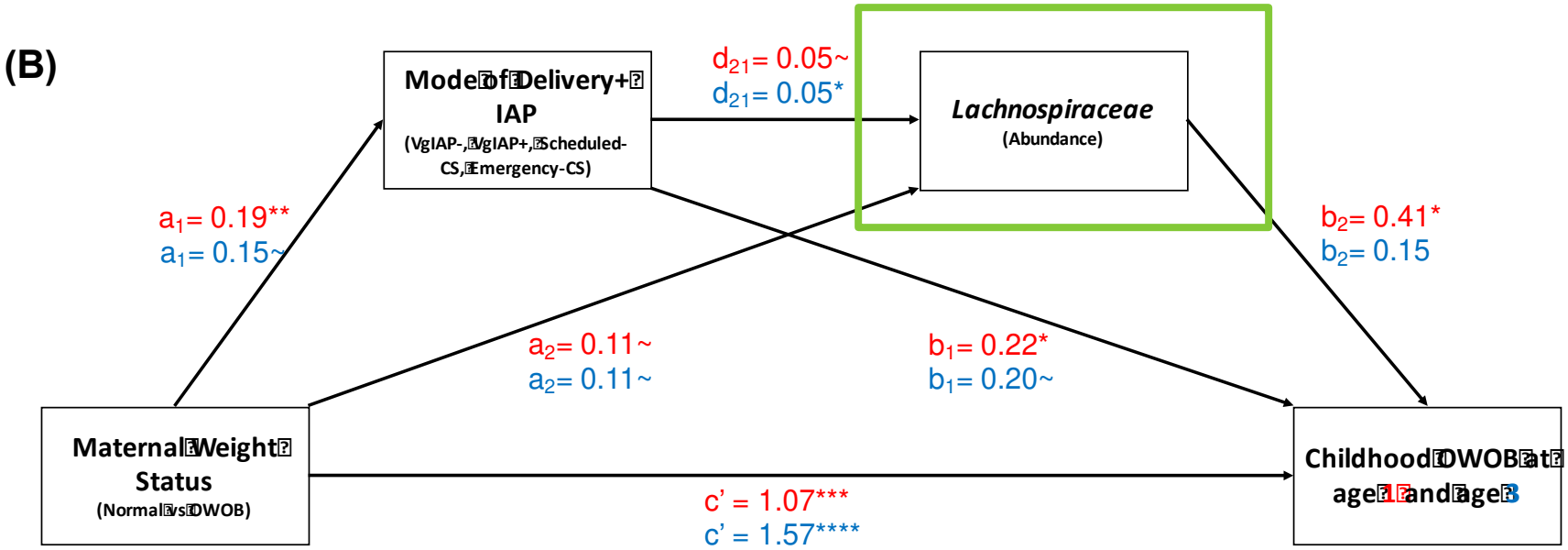
Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Birth mode and gut Lachnospiraceae JOINTLY mediate the association between maternal and child overweight



Bootstrap 95%CI for indirect effect ($a_1d_{21}b_2$)
[0.0001 , 0.0156]
[-0.0003 , 0.0095]

*~P<0.1, *P<0.05, **P<0.01, ***P<0.001, **** P<0.0001*

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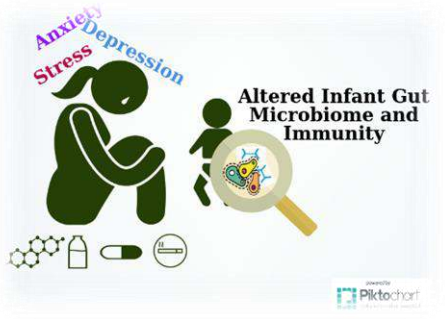
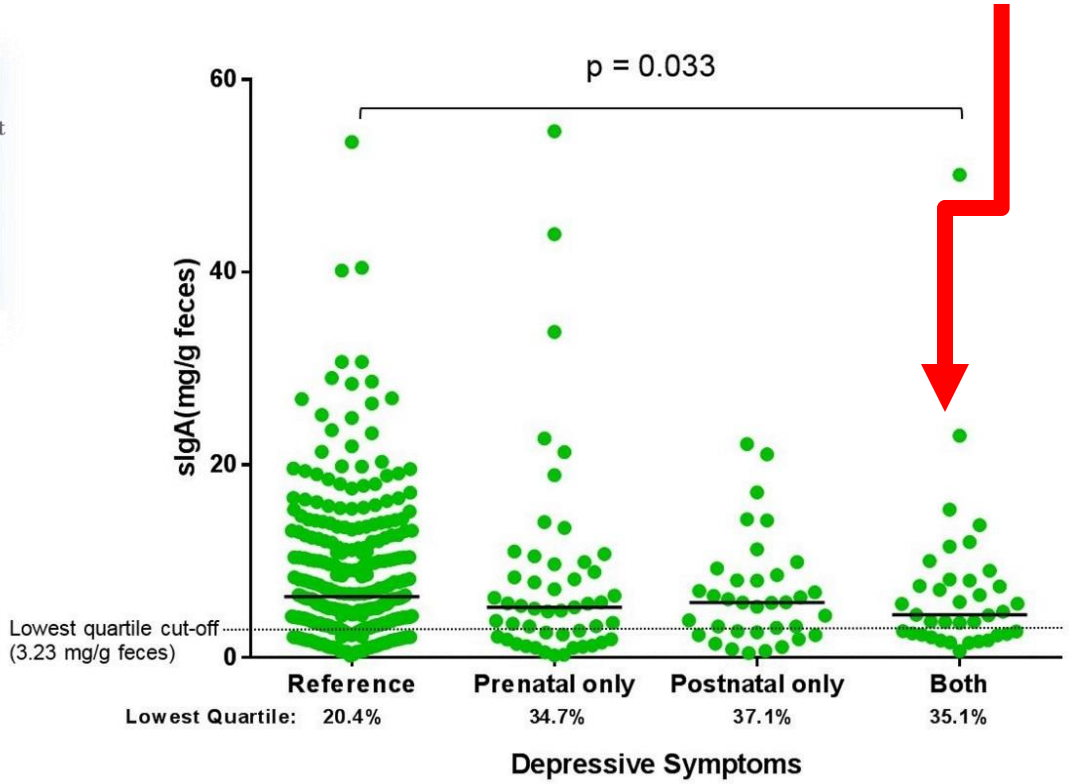
Tun et al. Roles of birth mode and infant gut microbiota in intergenerational transmission of overweight and obesity from mother to offspring. JAMA Pediatr

Fecal sIgA at 3 months was lower in infants of mothers with distress during pregnancy and afterwards

Kang et al. Maternal depressive symptoms linked to reduced fecal Immunoglobulin A concentrations in infants. Brain Behav Immun 2018;68:123-13

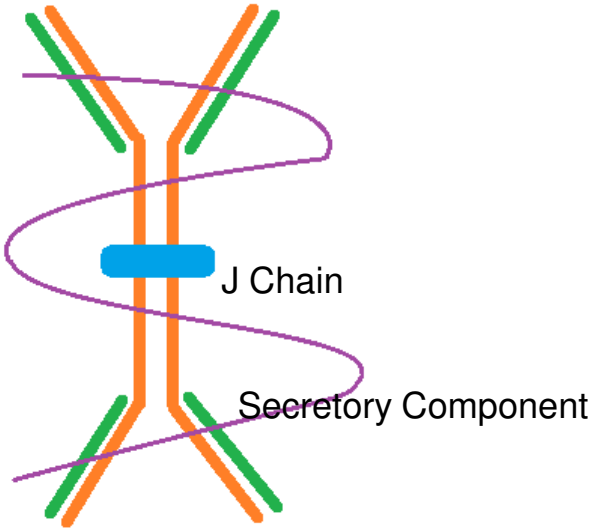




Secretory Immunoglobulin A measured in infant fecal samples at 3 months. It is critical to:

- Our gut immune defense system
- Immune system maturation in infants
- Induction of **oral tolerance** to food
- Reduced risk of **allergic diseases**

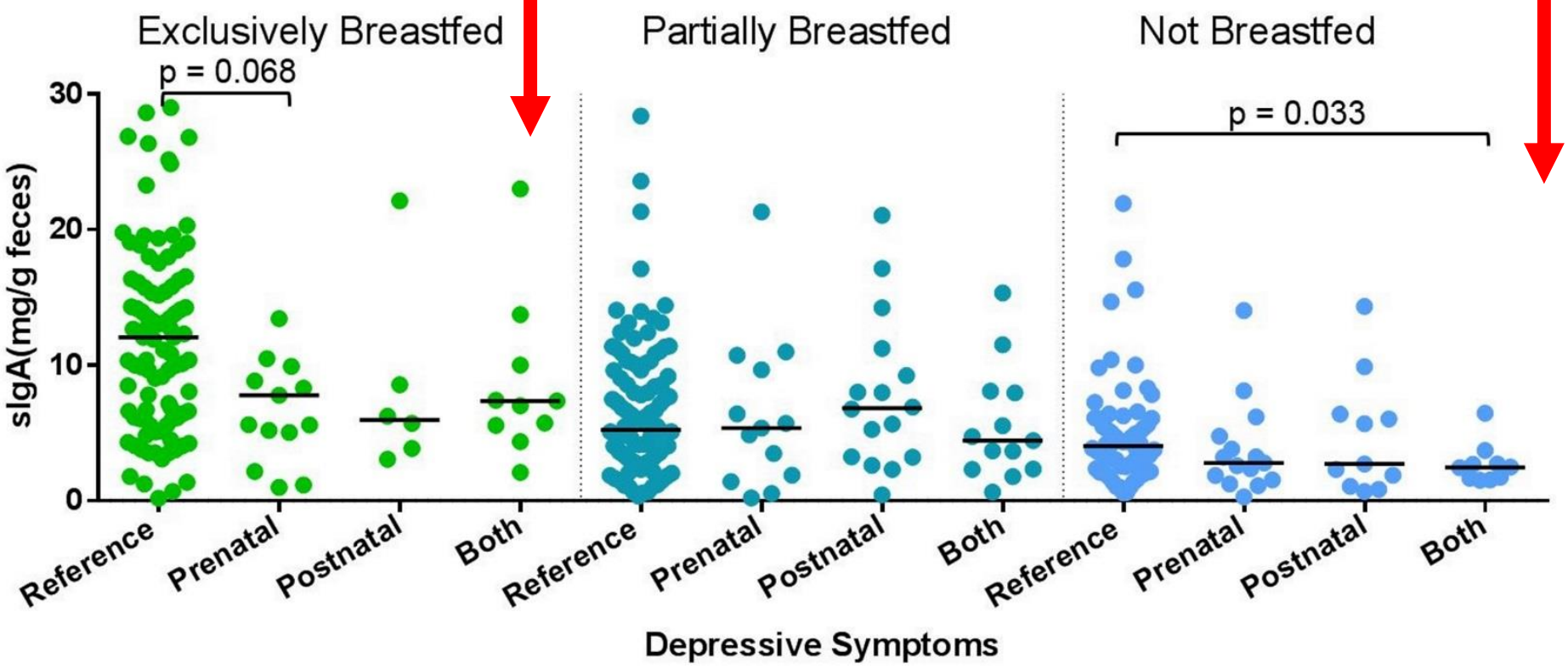
sIgA found in



Breast Milk

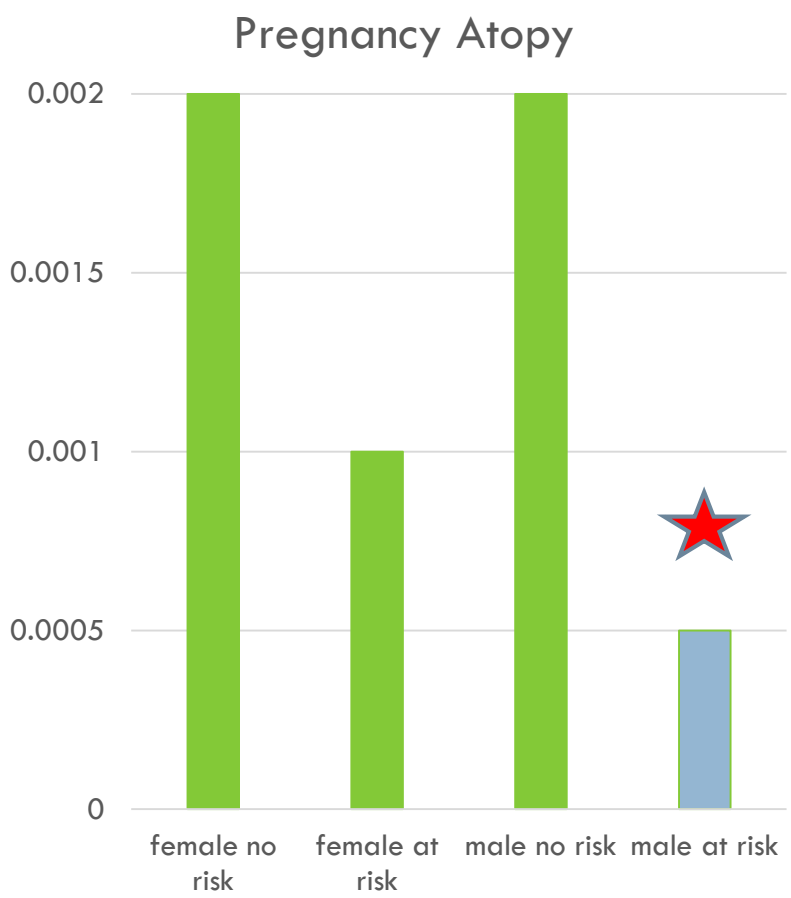
Bridgman et al. High fecal IgA is associated with reduced Clostridium difficile colonization in infants. Microbes Infect. 2016;18:543-9.

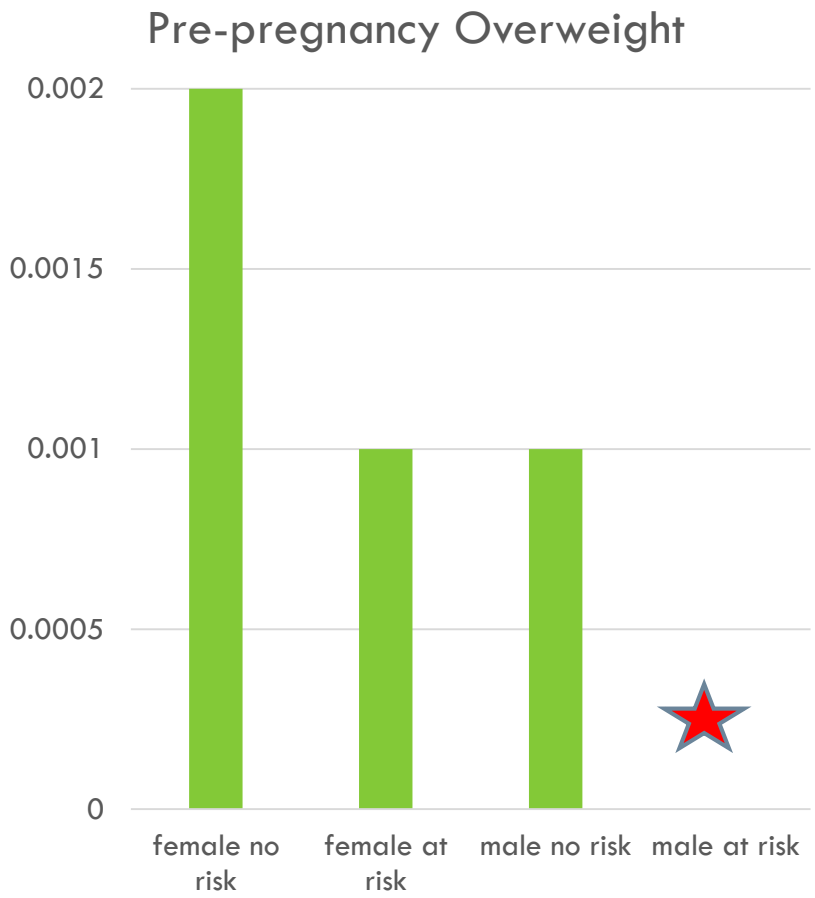
Especially among infants not breastfed but also seen in breastfed infants



Kang et al. Maternal depressive symptoms linked to reduced fecal Immunoglobulin A concentrations in infants. Brain Behav Immun 2018;68:123-13

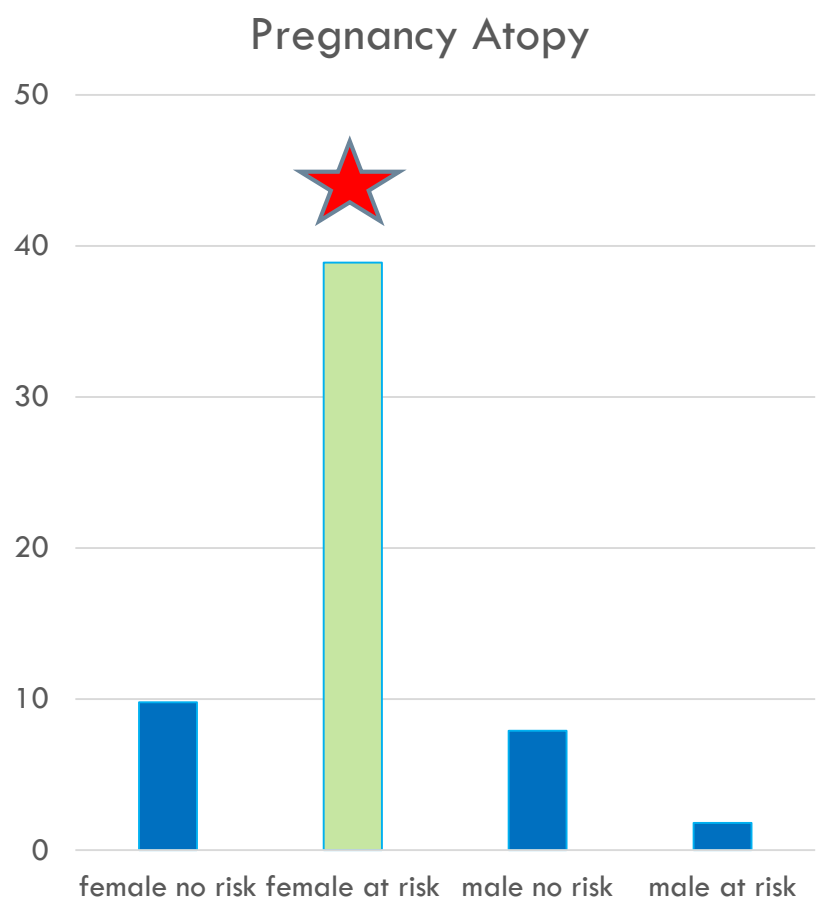
Independent of breastfeeding, ↓ fecal abundance of Lactobacillaceae at 3 months in male infants born to women with asthma during pregnancy (at risk for future asthma & overweight)

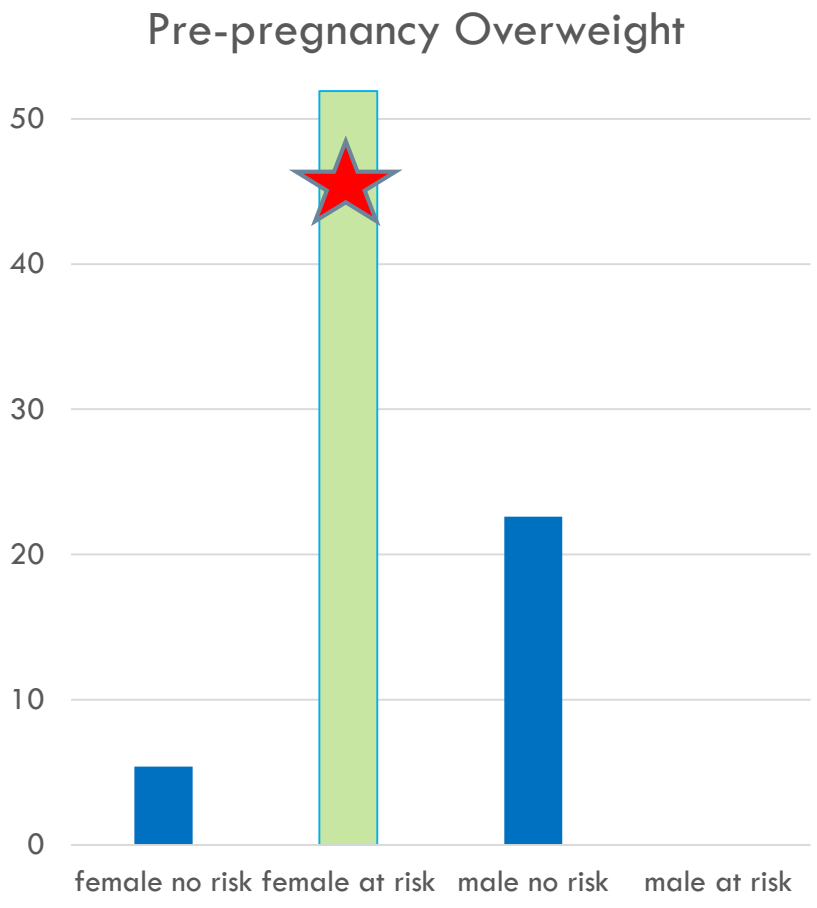




Koleva et al. Sex-specific impact of asthma during pregnancy on infant gut microbiota. Eur Resp J 2017;50

↑ fecal abundance of Bacteroidaceae at 3 months in female infants born to women with asthma during pregnancy (at risk for future asthma & overweight)





Koleva et al. Sex-specific impact of asthma during pregnancy on infant gut microbiota. Eur Resp J 2017;50

↓ BACTEROIDETES + ↑ ENTEROBACTERIA = FOOD SENSTIZATION

Azad et al. Infant gut microbiota and food sensitization: associations in the first year of life. Clin Exp Allergy 2015; 45(3): 632-643.

Cited in: US National Academies of Sciences, Engineering, and Medicine. 2016. Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management and Public Policy. Washington DC

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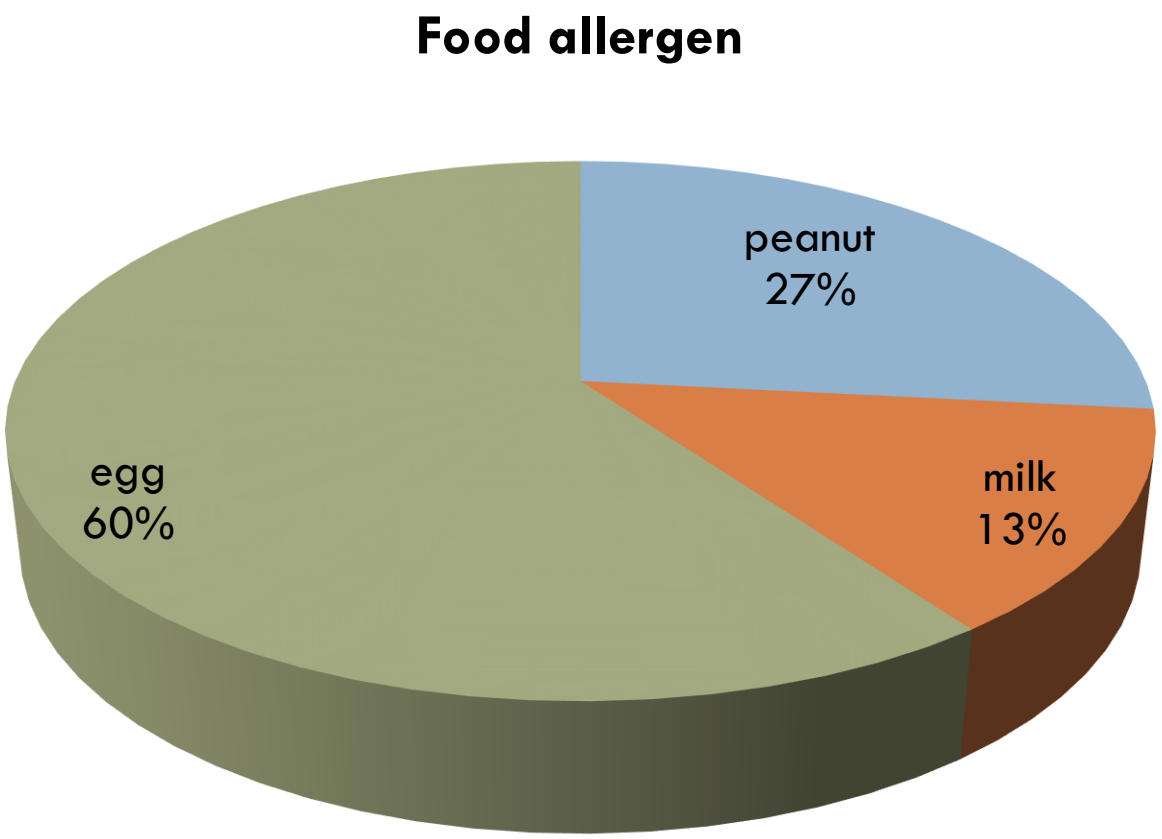
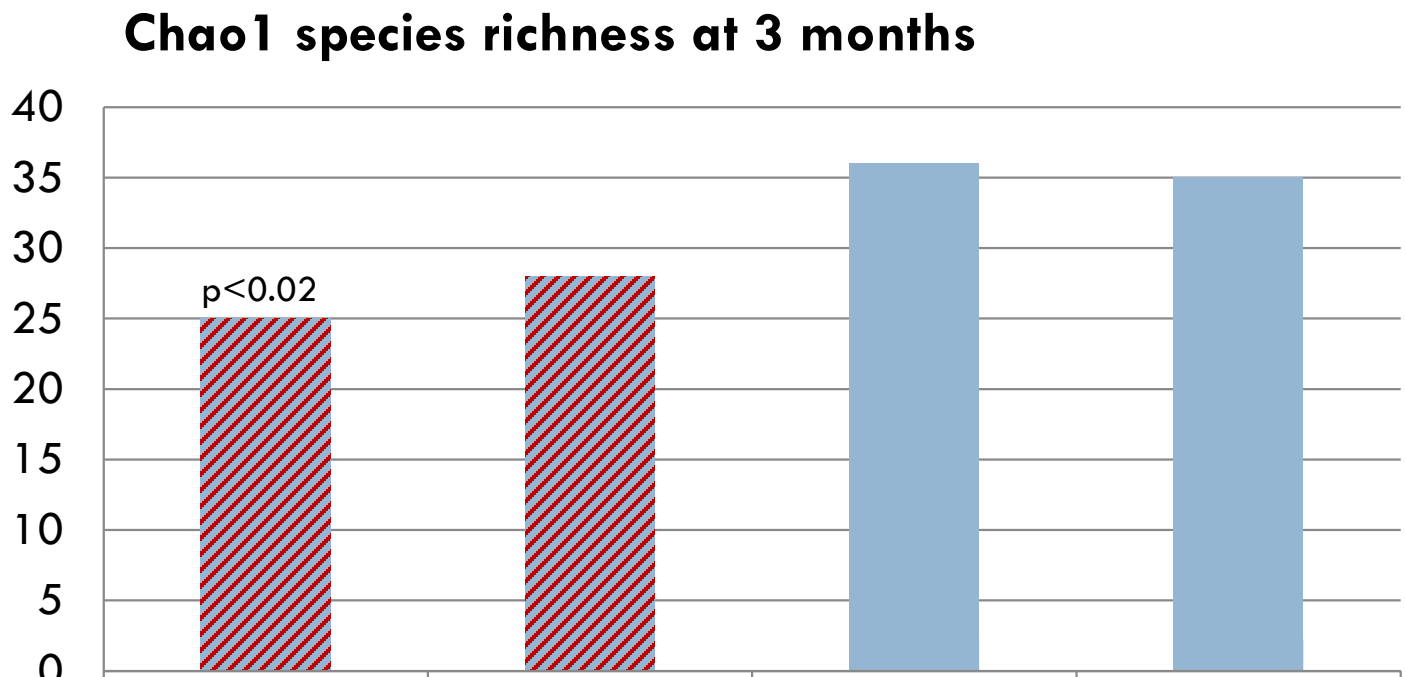
SYNERGY IN MICROBIOTA RESEARCH

Canadian Healthy Infant Longitudinal Development (CHILD) cohort

Food sensitization in infants is a 2-fold risk for asthma & allergic disease !

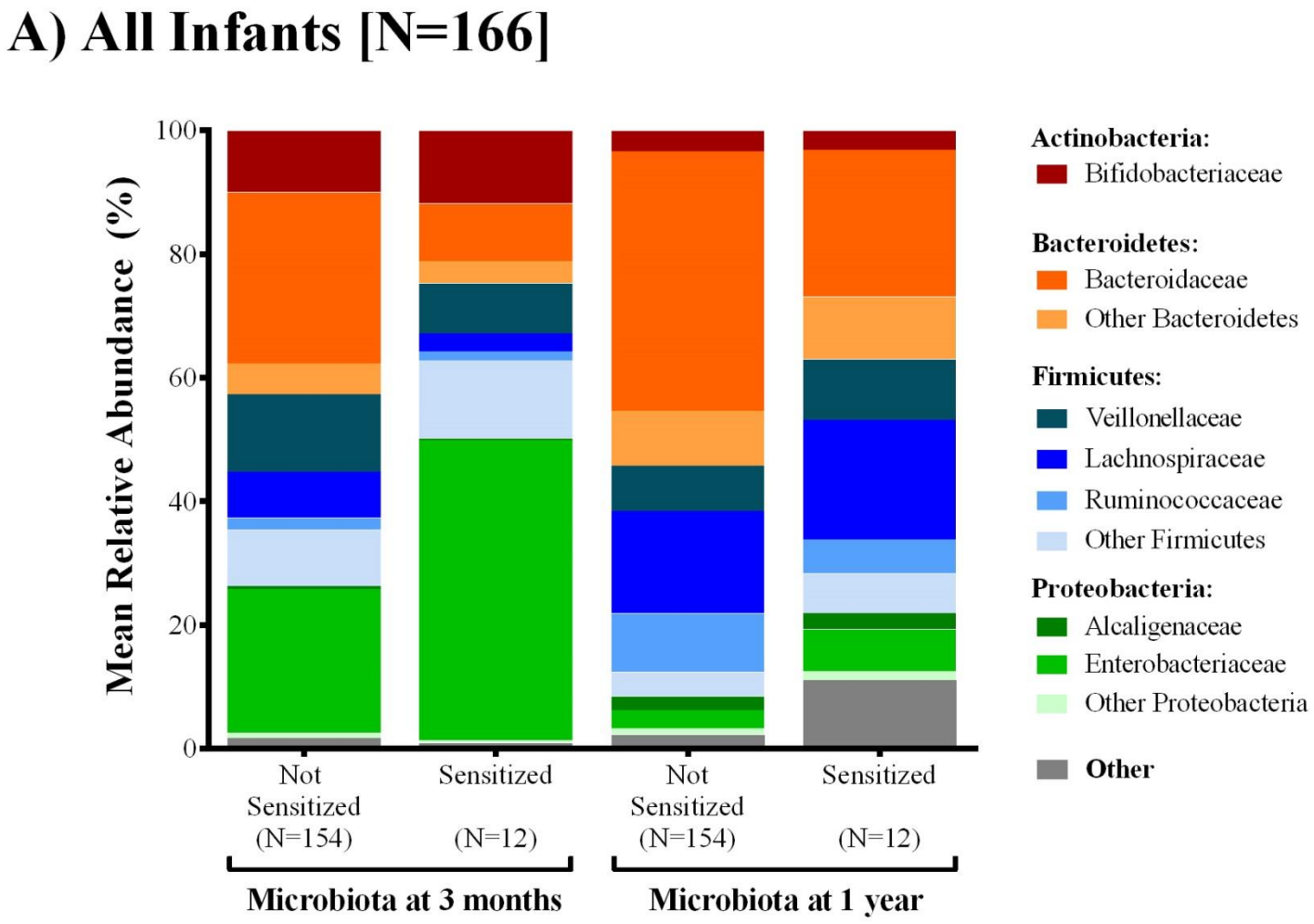


Species richness was significantly lower at 3 months but not 1 year in sensitized infants



	Sensitized-3 mo (n=12)	Not sensitized-3 mo (n=154)	Sensitized-1 yr (n=12)	Not sensitized-1 yr (n=154)
Shannon diversity	1.6	1.9	2.3	2.2
Chao1 richness	25	28	36	35

Fewer Bacteroidaceae/more Enterobacteria (↑E/B) in food sensitized infants at 1 year



E/B ratio and low species richness associations with sensitization are independent of each other

Likelihood of Sensitization at 1 Year

Model Adjustments	Microbiota at 3 months		Microbiota at 1 year	
Microbiota Measure	aOR (95% CI)		aOR (95% CI)	
Mutual adjustment for microbiota only				
E/B Ratio (per quartile increase)	2.02	(1.07 - 3.80)*	4.14	(1.54 - 11.11)*
Chao1 Richness (per quartile increase)	0.45	(0.23 - 0.87)*	-	-
Adjusted for any antibiotic exposure before sample				
E/B Ratio (per quartile increase)	2.13	(1.10 - 4.13)*	4.03	(1.50 - 10.82)*
Chao1 Richness (per quartile increase)	0.45	(0.23 - 0.87)*	-	-
Adjusted for caesarean delivery				
E/B Ratio (per quartile increase)	2.21	(1.13 - 4.31)*	4.35	(1.57 - 12.11)*
Chao1 Richness (per quartile increase)	0.42	(0.21 - 0.85)*	-	-
Adjusted for exclusive breastfeeding at 3 months				
E/B Ratio (per quartile increase)	1.80	(0.93 - 3.50)	4.02	(1.48 - 10.88)*
Chao1 Richness (per quartile increase)	0.46	(0.24 - 0.89)*	-	-

Acknowledgements

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Data & Sample Collection
CHILD Study Staff & Families

Project Management
Sarah Bridgman, Sylva Donaldson

DNA Extraction, Sequencing & Bioinformatics (Toronto)
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Trainees & Research Assistants
Hein Tun, Liane Kang, Usha Rai, Brittany Matenchuk, Kelsea Drall

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SYMBIOTA
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LOOKING FOR NEW POSTDOCS

The Ladies of Canmore

www.in-flame.org/2018-meeting

CHILD: Microbiota profiling methods

- Fecal samples at 3 months from 1000 full-term infants sequenced with MiSeq at V4
- Sequences clustered at 97% similarity against the Greengenes database (closed-picking) to identify Operational Taxonomic Units (OTUs)
- Rare OTUs (<0.0001relative abundance) excluded
- Data were rarefied to 15,000 sequences per sample
- OTU relative abundance and diversity indices (Chao1, Shannon) were generated by the QIIME program