Necrotizing Enterocolitis: An Update

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Agenda

- Definition of “NEC”: Can we focus on a “Classic NEC”?
- Dysbiosis and NEC: Relation to Pro and Anti-Inflammatory mediators.
- Are neonatologists causing dysbiosis in preterms?
- The Future

Historical Perspective: Being led astray: 50 years---not much progress

- Lumping of several diseases called "NEC" into the same data set.
- Animal models that do not represent the disease we see in human preterms.
- Narrow focus on individual pathways rather than systems.

Gestational Age and NEC

Many NICUs “never see NEC”

Incidence of NEC increases by 3% for every 250 gram decrement: Fitzgibbons, et. al, J Peds Surg. 2006.

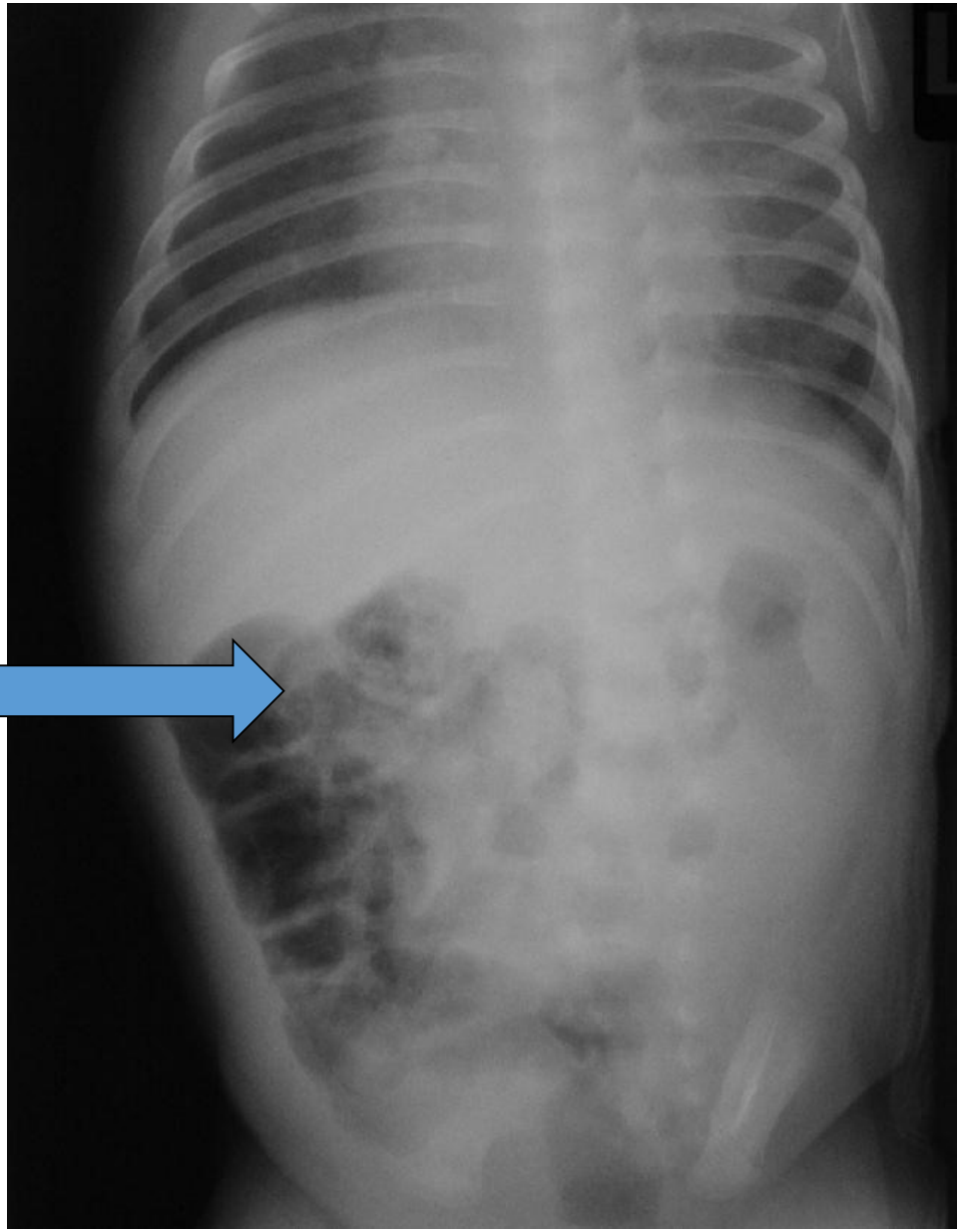
NEC in babies between 1250-1500grams<1%,
But 500-750 grams is 9-12%.

If NICU A sees 10 babies <750 grams/year=1 baby with NEC.
If NICU B sees 50 babies <750 grams/year=5 X as many babies with NEC as NICU A.

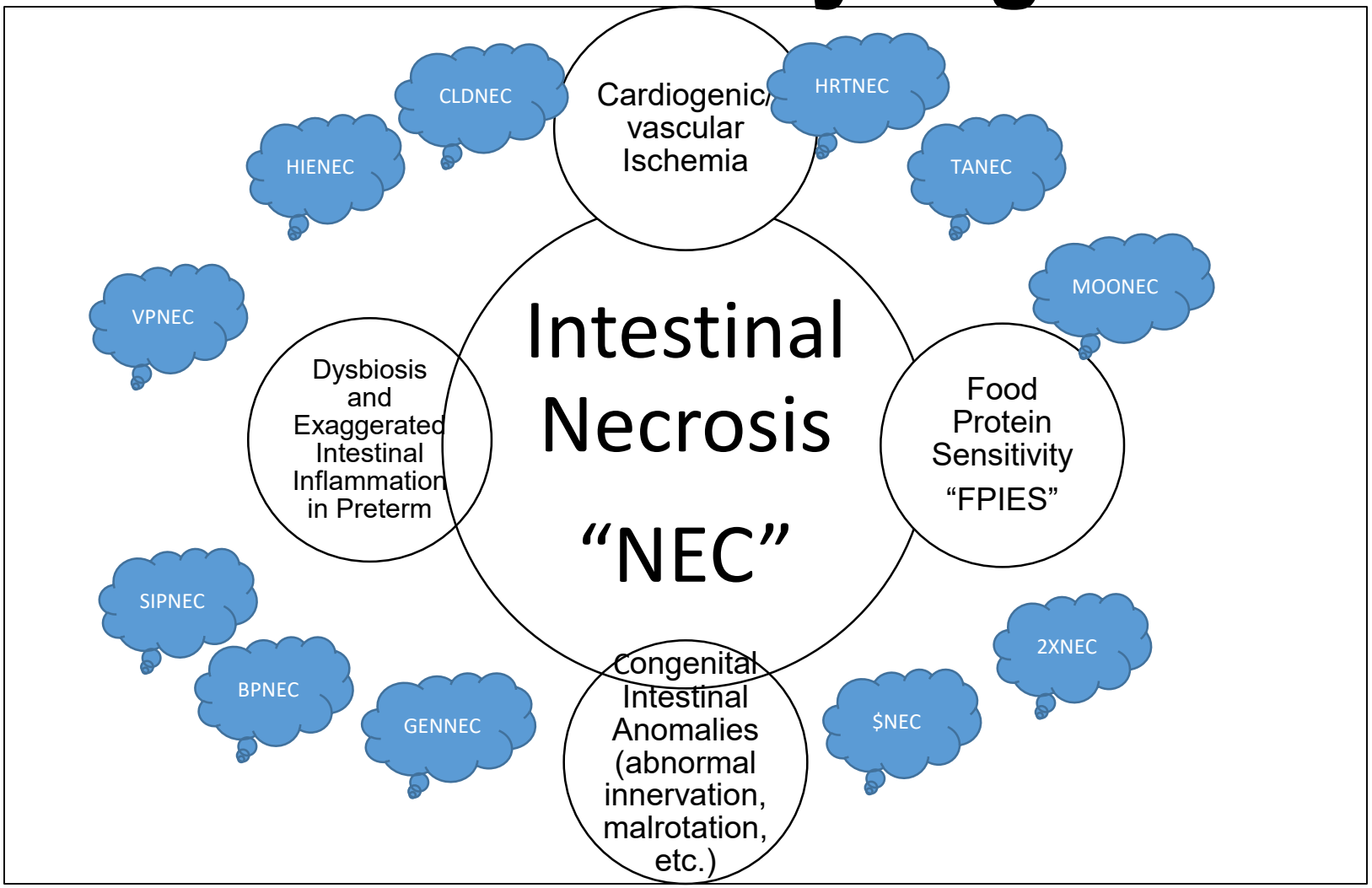
Is there a Clear Definition of NEC? Bells is Broken

- Stage 1-Too non-specific and the term should not be used.
- Stage 2-Radiographic signs can be “fuzzy”.
- Stage 3- Free air on radiograph could signify intestinal necrosis or Spontaneous Intestinal Perforation (SIP)

“Poopatosis”

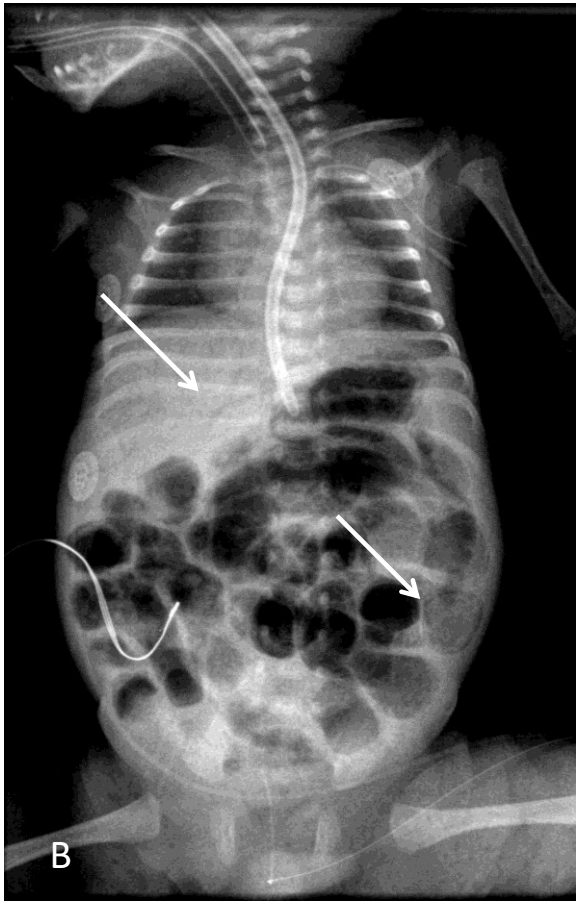


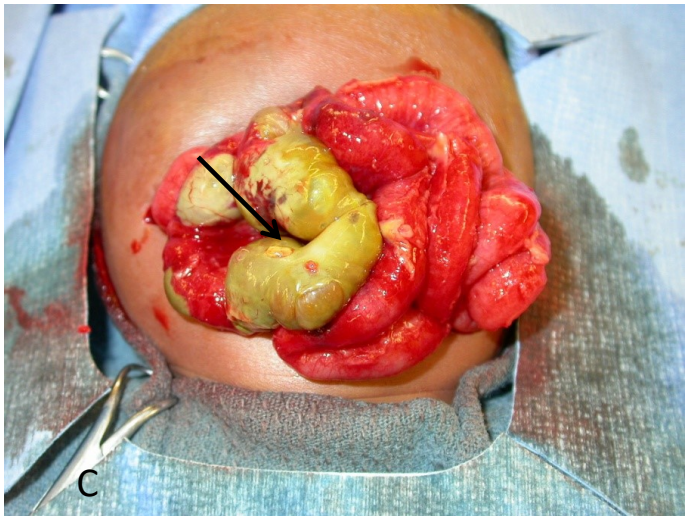
More than one disease or one disease with many origins?



Adapted from Gordon, PV. Et al.Seminars in Perinatology, 2017

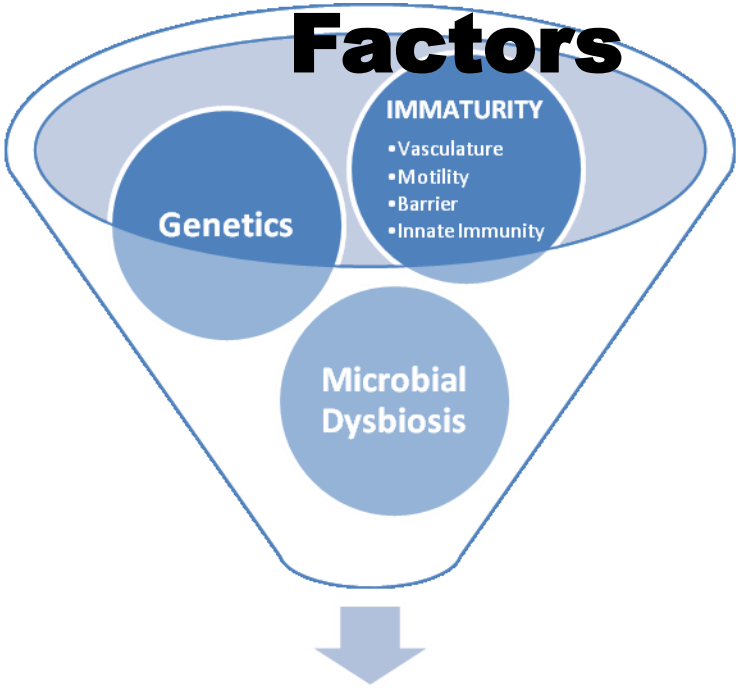
“Classic” NEC





Neu, J. and Walker , W. A. New England Journal of Medicine, Jan. 2011

What Causes Classic "NEC"? Some Factors



NEC

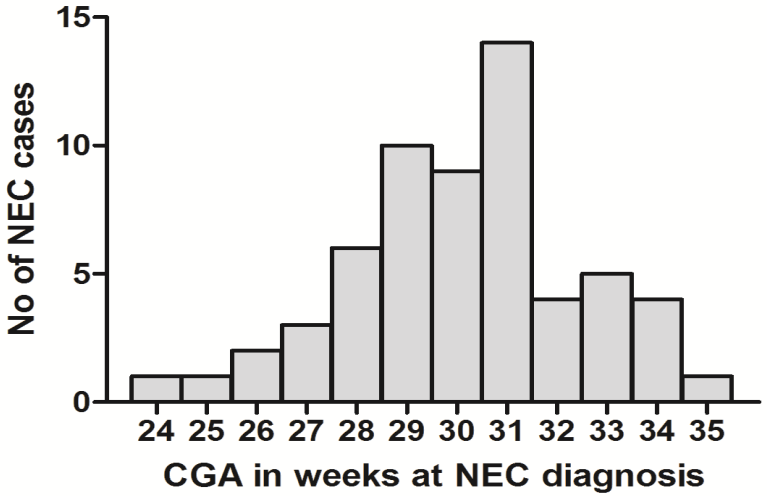
Where's Hypoxia-Ischemia and Feeding?

Rat model of "NEC".

Mean Gestational Age at NEC Diagnosis

23 week preterm

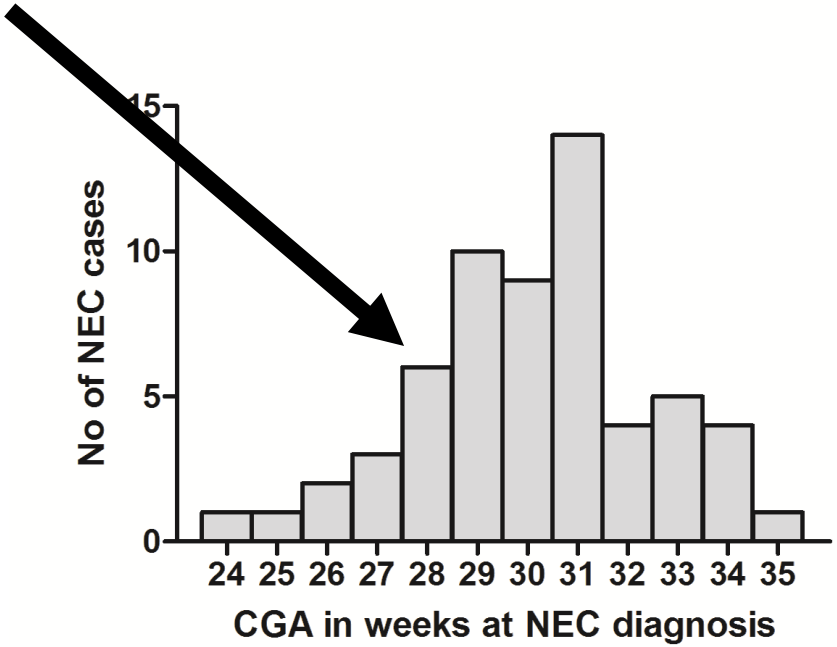
29 week preterm



Pammi, M. et al. Microbiome, 2017

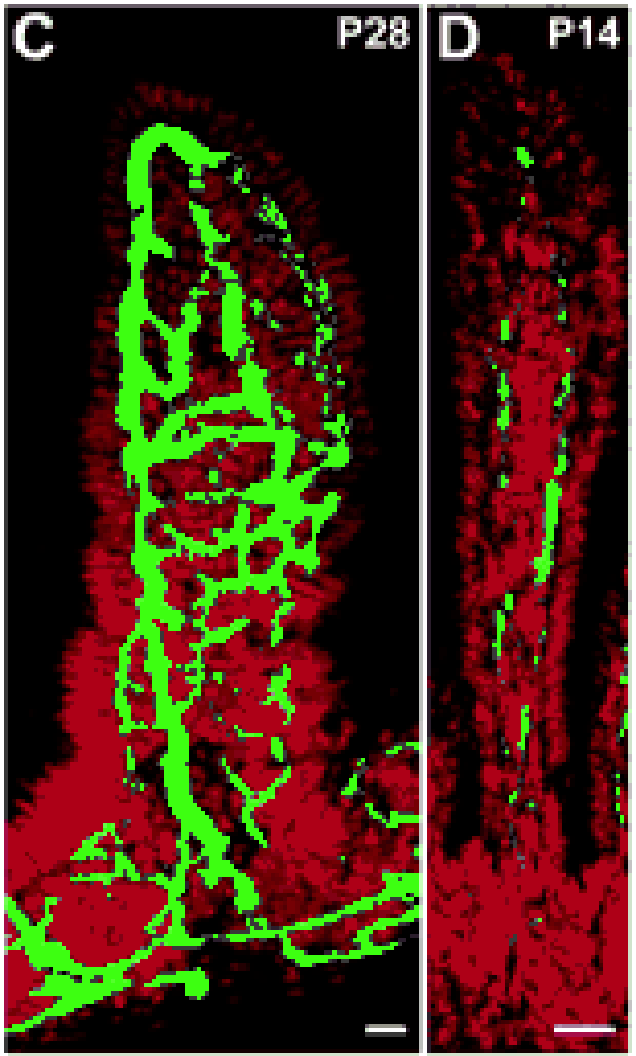
Mean Gestational Age at NEC Diagnosis

- Microvasculature Changes?
- TLR Developmental Pattern?
- Microbiota changes?



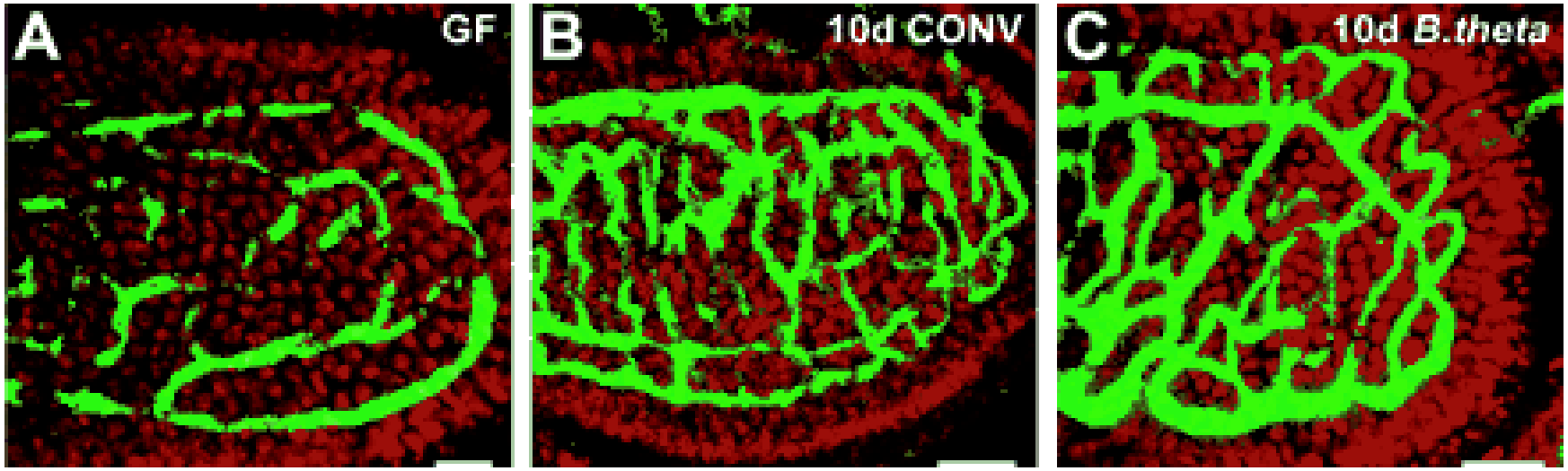
Pammi, M. et al. Microbiome. 2017 Mar 9;5(1):31

Development of Intestinal Angiogenesis With Microbes



Stappenback, Hooper and Gordon, *PNAS*, 2002

Development of Intestinal Angiogenesis With Microbes

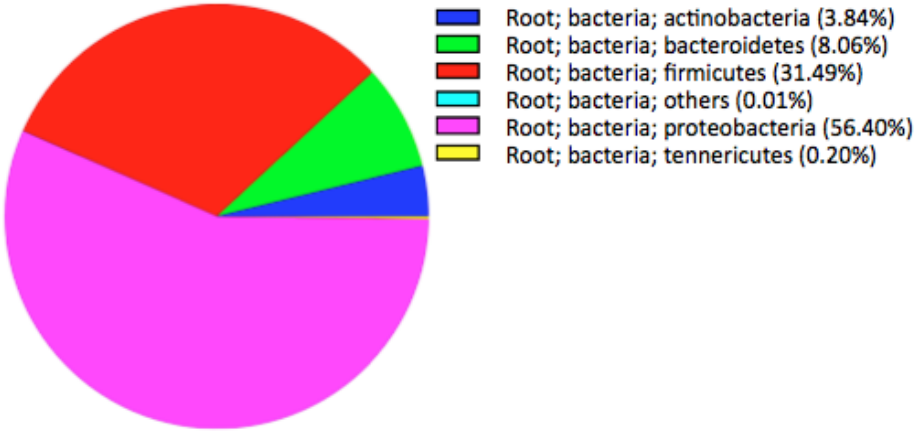


Stappenback, Hooper and Gordon, *PNAS*, 2002

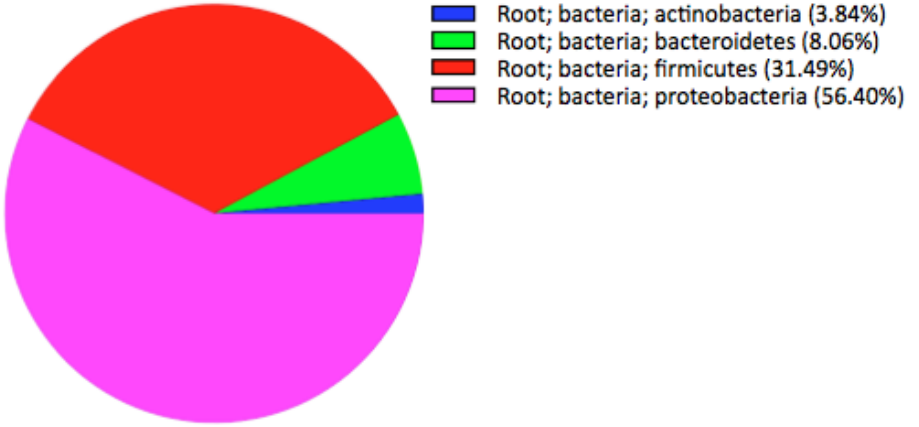
FECAL MICROBIOTA: NEC

Mai V, Young C. PLOS One, May 2011

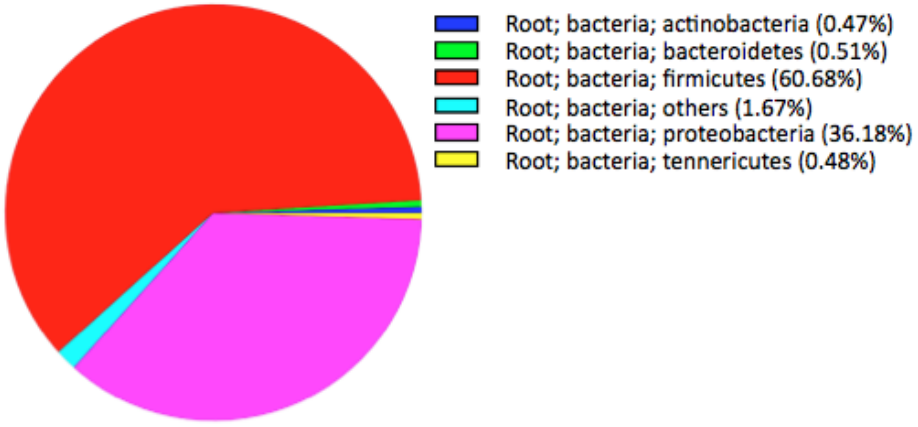
Controls, one week before diagnosis



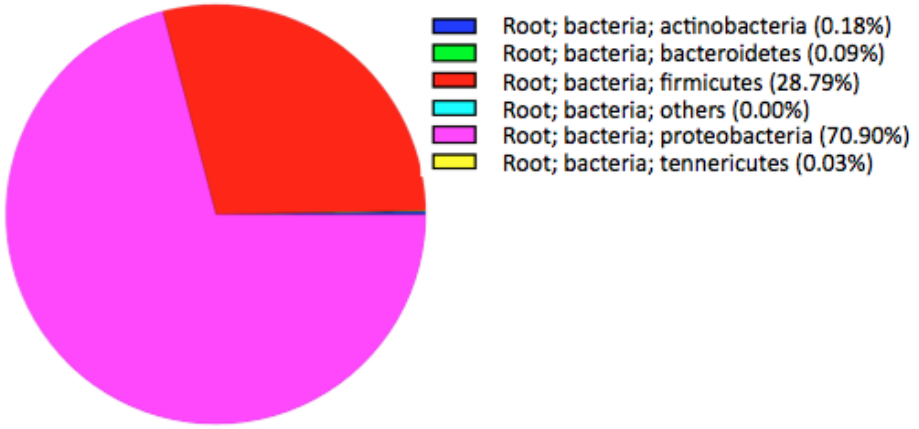
Controls, <72h of diagnosis



Cases, one week before diagnosis



Cases, <72h of diagnosis



Microbial Shift Prior to NEC

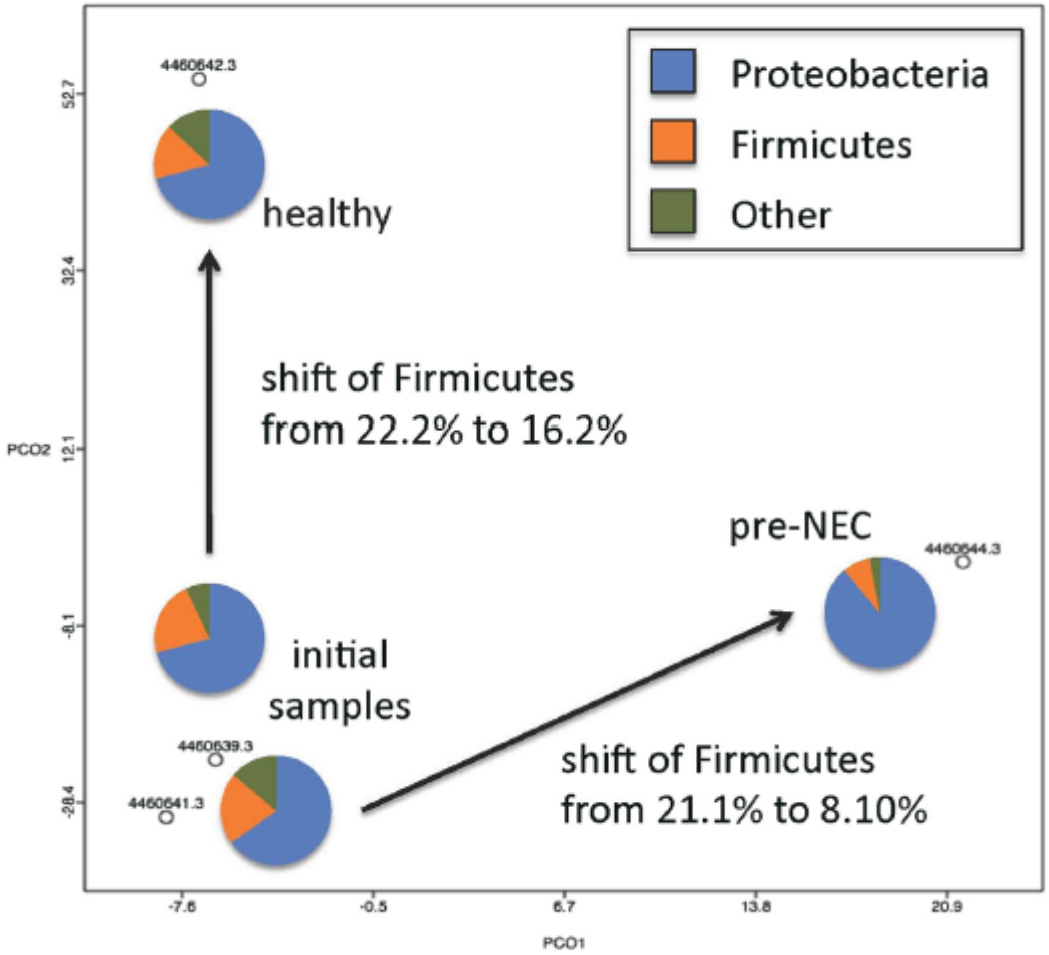
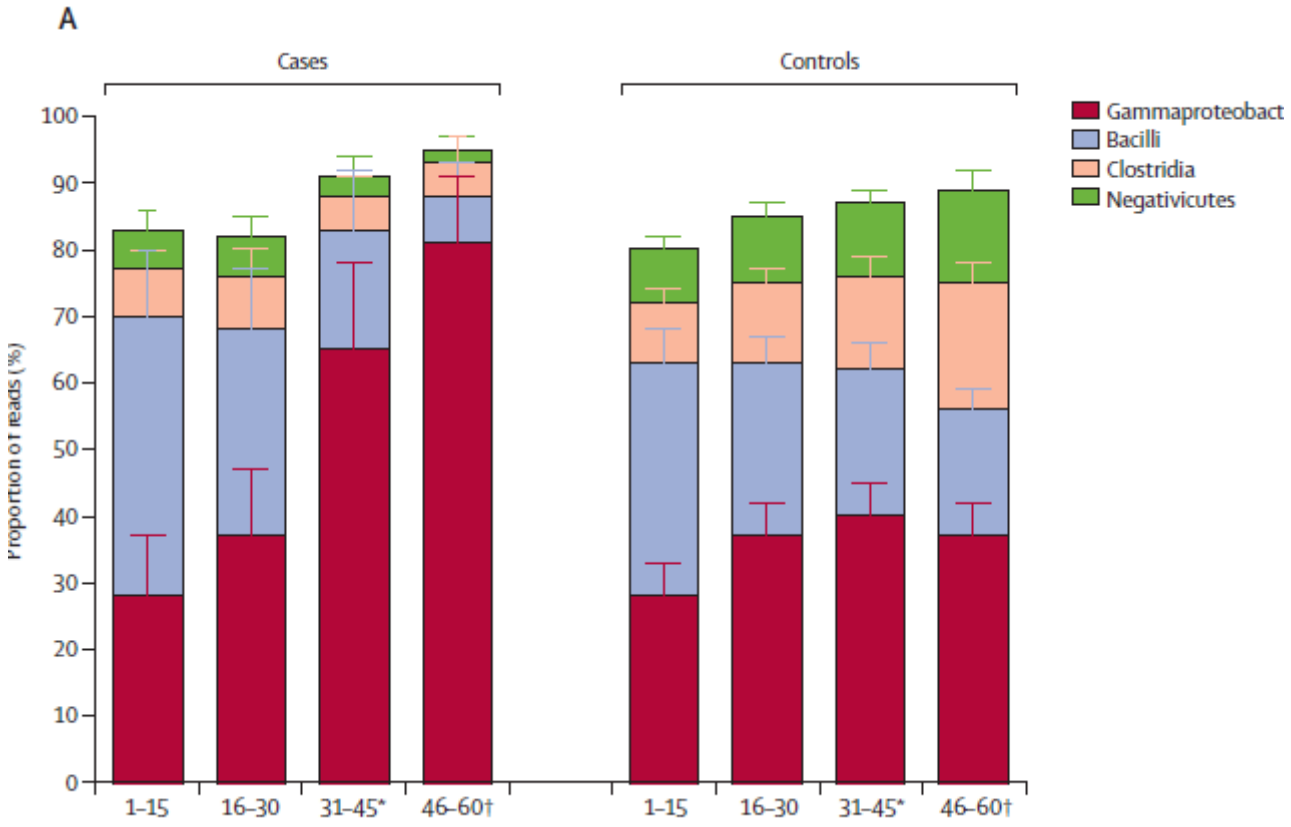


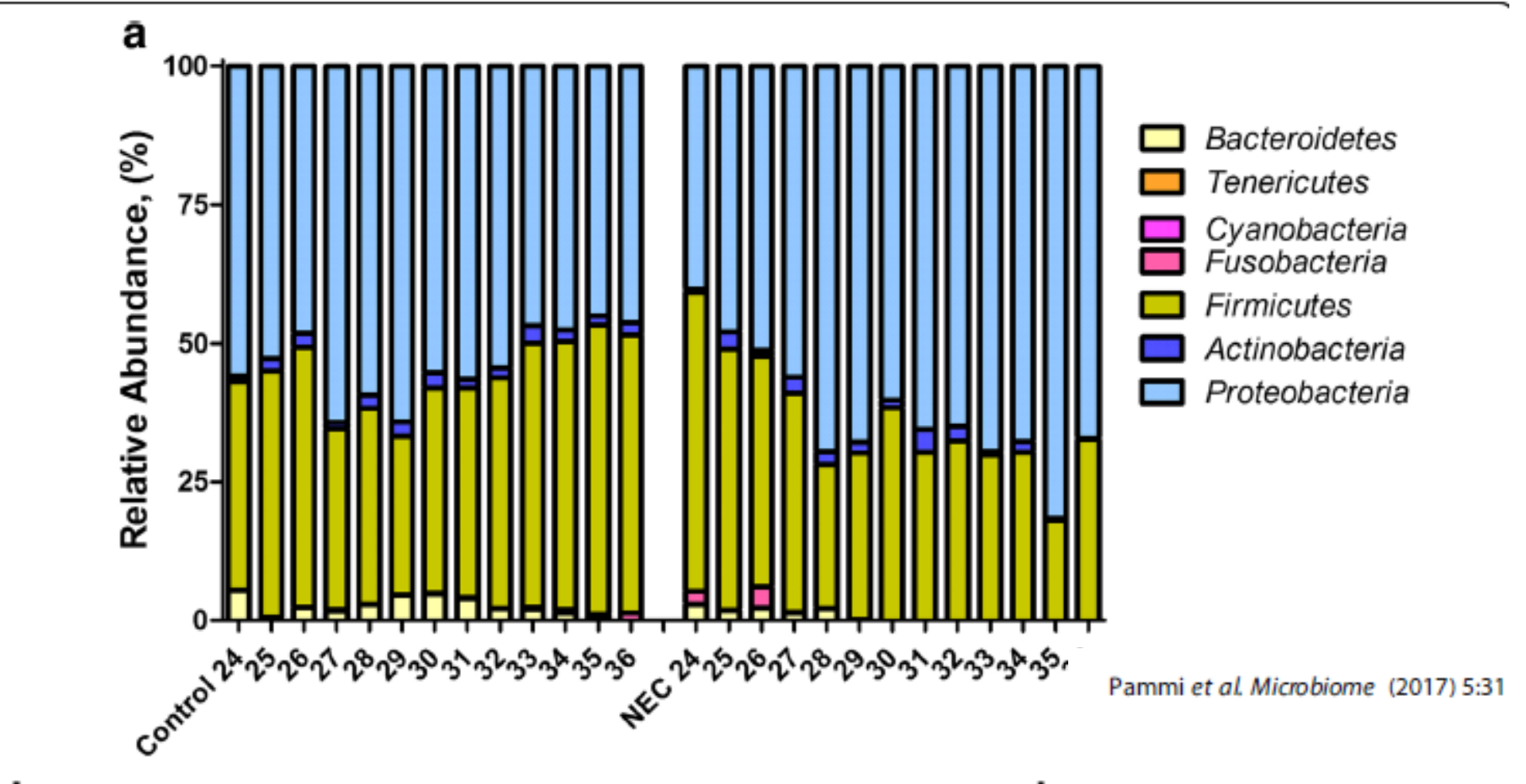
Figure 4 Analysis of microbial communities by shotgun metagenomics between two weeks of life and NEC diagnosis demonstrate functional distinction. Shotgun metagenomes generated from twin patients at times prior to NEC diagnosis (only one of the twins went on to be diagnosed with NEC; labeled 'pre-NEC'). An expansion of the Proteobacteria is noted in the patient that went on to develop NEC.

From Claud, E. et al . Microbiome, 2013

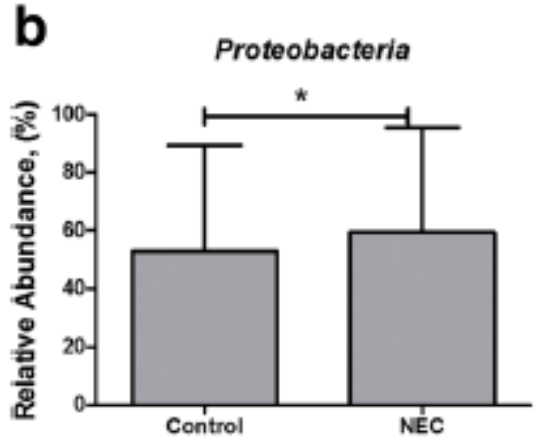
Abundance of Gamma-Proteobacteria

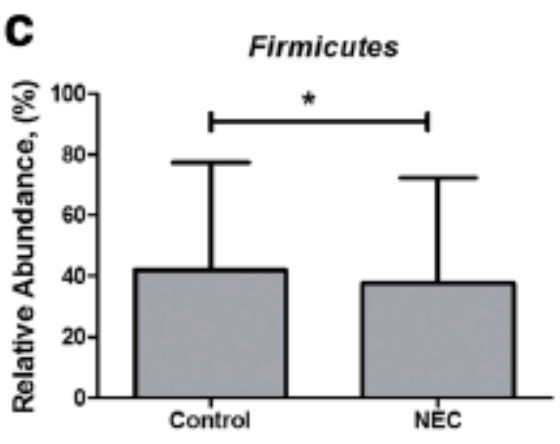


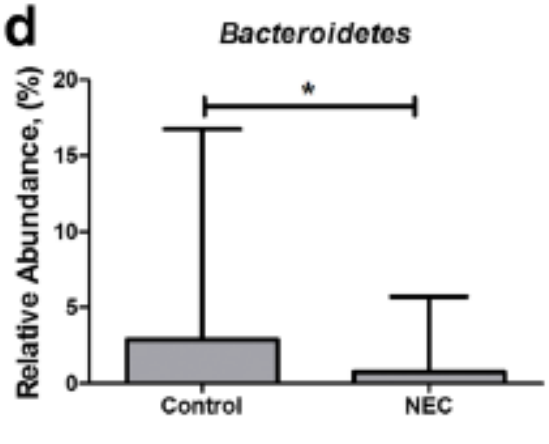
Warner, B. et al. Lancet March 8,2016



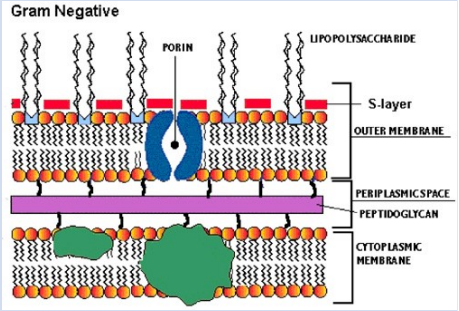
Pammi *et al. Microbiome* (2017) 5:31



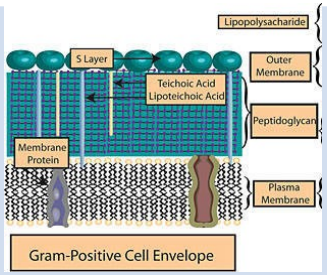




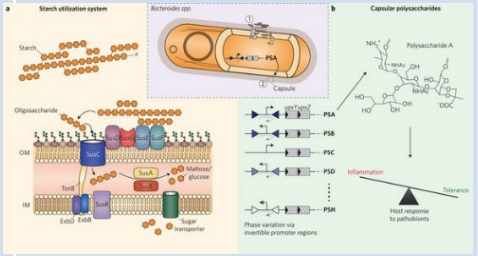
Comparison of three major phyla: Proteobacteria, Firmicutes and Bacteroidetes

Phylum	Gram Staining	Functional Relationship	Comment
Proteobacteria	Gram negative	High Lipopolysaccharide (LPS) content in cell wall. Abundance of Proteobacteria increased prior to exacerbations of inflammatory bowel diesease. Strong stimulator of TLR4. E. Coli, Klebsiella and Pseudomonas are representatives.	

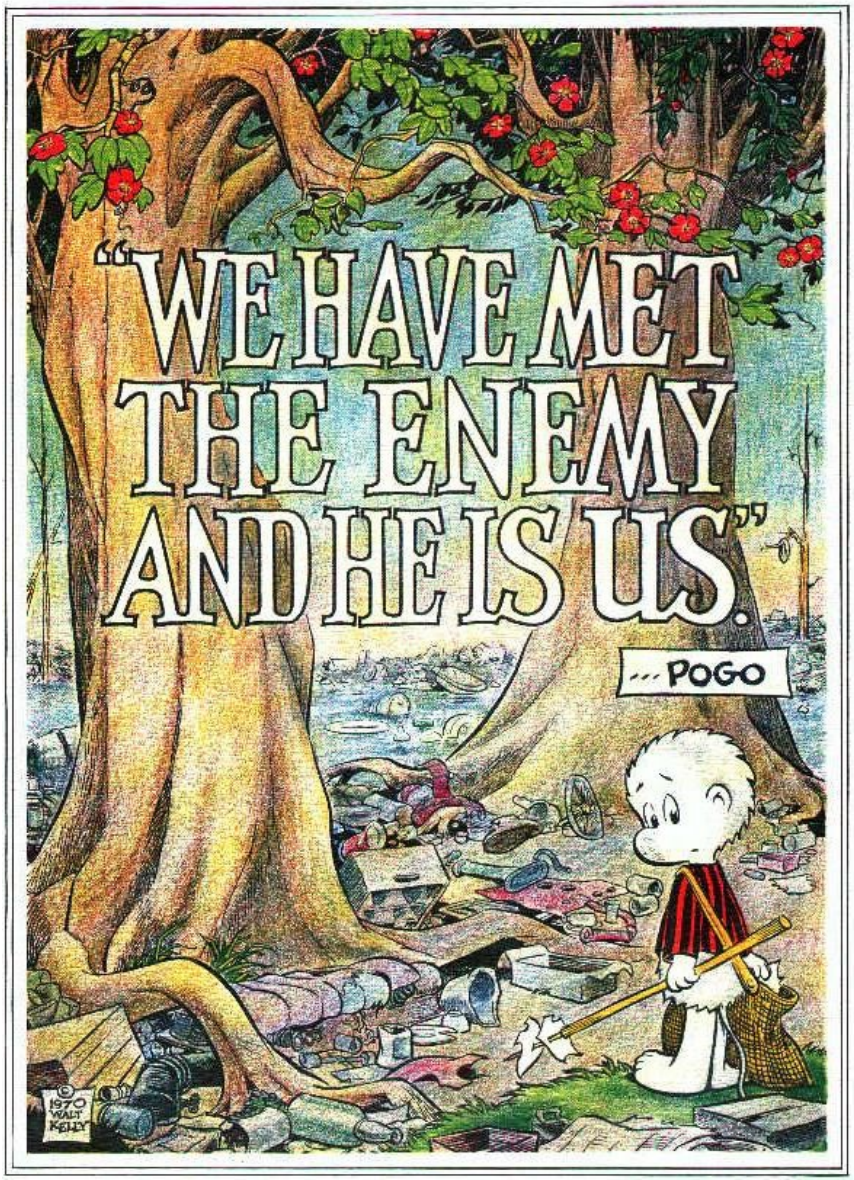
Comparison of three major phyla: Proteobacteria, Firmicutes and Bacteroidetes

Phylum	Gram Staining	Functional Relationship	Comment
Firmicutes	Gram positive	Lactobacilli are a common class of the Firmicutes phylum. Have high lipoteichoic acid in the cell wall, but low LPS. Have excellent capacity for energy harvest. Produce butyrate in high quantities. Butyrate is a major fuel for colonocytes and important for maintenance of tight junctions.	

Comparison of three major phyla: Proteobacteria, Firmicutes and Bacteroidetes

Phylum	Gram Staining	Functional Relationship	Comment
Bacteroidetes	Gram negative, anaerobic, rod shaped bacteria.	Involved in fermentation of carbohydrates (propionate and acetate producers), utilization of nitrogenous substances, and biotransformation of bile acids. Bacteroides fragilis is a representative. The immunomodulatory molecule, polysaccharide A (PSA), of *B. fragilis* mediates the conversion of $CD4^+$ T cells into $Foxp3^+$ Treg cells that produce IL-10 during commensal colonization. PSA is not only able to prevent, but also cure experimental colitis in animals. Propionic acid is also a strong inducer of the Foxp3+ R regulatory pathway.	

Do Common Neonatal Practices Cause NEC?



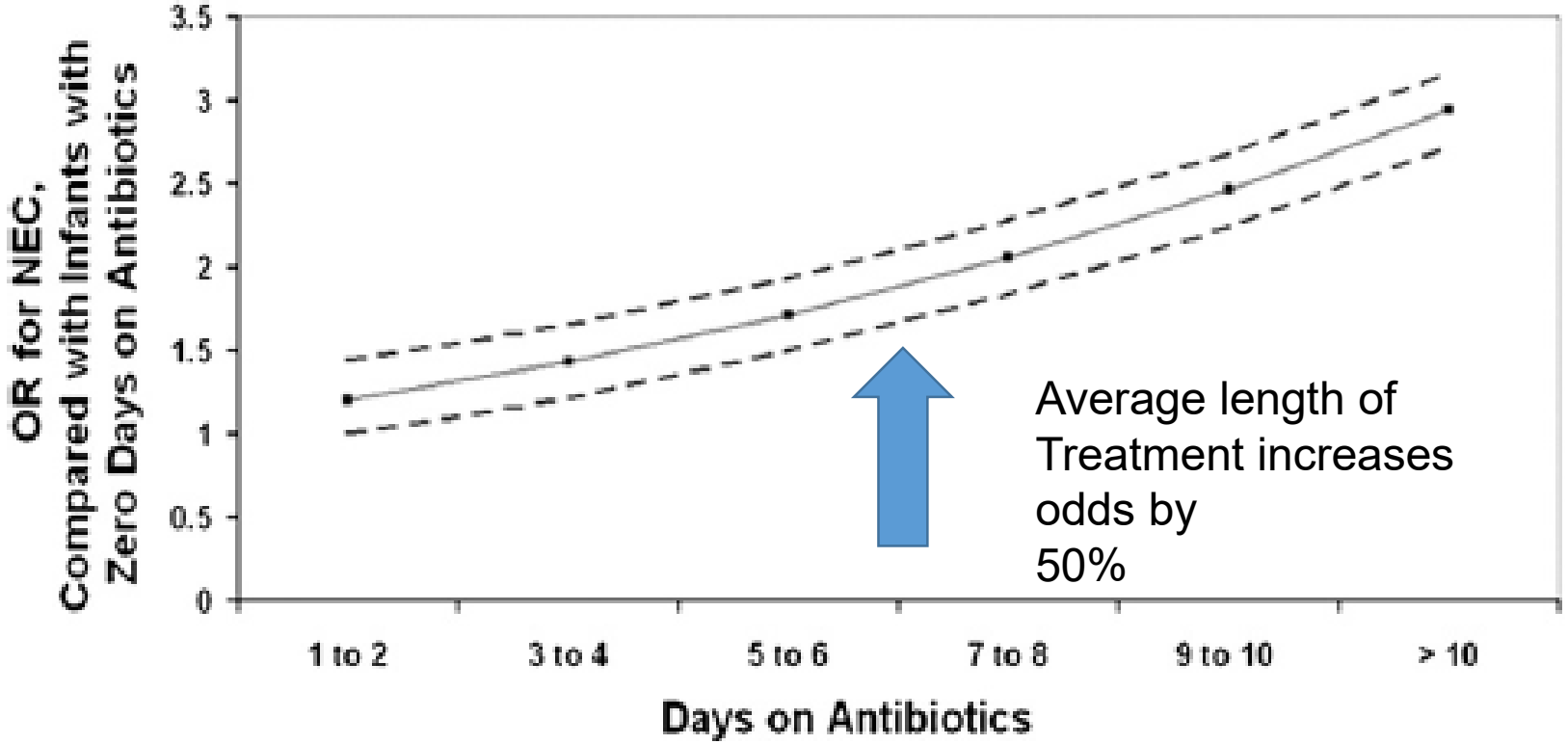
Most Commonly used Drugs in the NICU: Majority of VLBW infants are Exposed to Antibiotics

Top 10 Medications Prescribed in the NICU

Medication Name	Frequency, *A*
Ampicillin	186 799
Gentamicin	171 388
Ferrous sulfate	90 152
Vitamin (multivitamin)	64 329
Cefotaxime	55 455
Caffeine citrate	48 814
Furosemide	47 278
Vancomycin	44 218
Beractant (Survanta)	36 410
Metoclopramide	27 541

Odds Ratio of NEC with Increased Days on Antibiotics

Alexander, V.N. J. Pediatrics, Sept. 2011



Gastric Acid Inhibition

Ranitidine is Associated With Infections, Necrotizing Enterocolitis, and Fatal Outcome in Newborns

WHAT'S KNOWN ON THIS SUBJECT: Although still off-label for newborns, the use of inhibitors of gastric acid secretion continues to increase. Acid-suppressive drugs could facilitate the onset of infections in adults and children. Evidence for efficacy is weak in newborns, particularly if preterm.

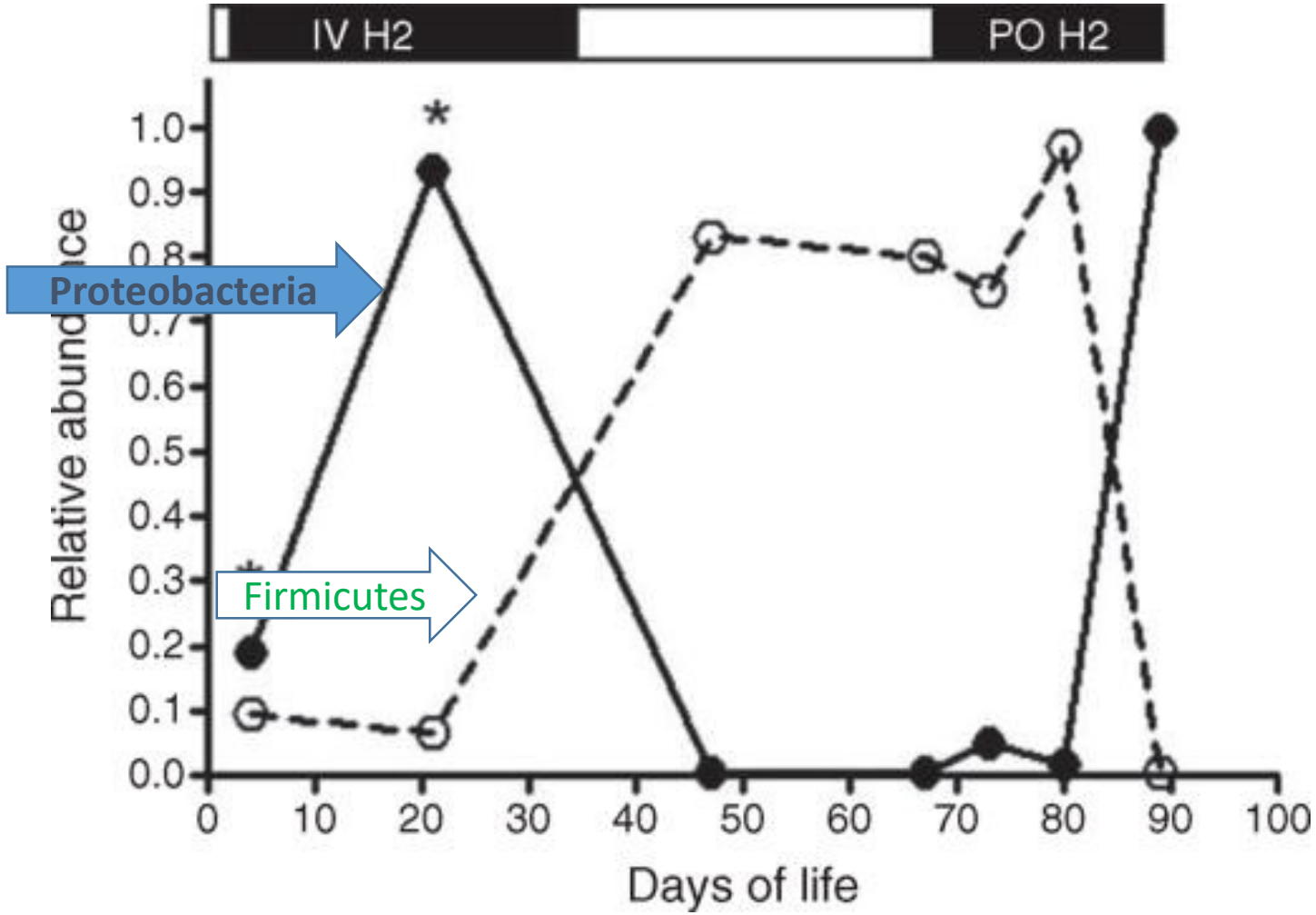
WHAT THIS STUDY ADDS: This is the first prospective study demonstrating an association between the use of ranitidine and infections, necrotizing enterocolitis, and fatal outcome in very low birth weight newborns. Caution is advocated in using ranitidine in newborns.

AUTHORS: Gianluca Terrin, MD, PhD,[a] Annalisa Passariello, MD, PhD,[b,c] Mario De Curtis, MD, PhD,[d] Francesco Manguso, MD, PhD,[e] Gennaro Salvia, MD,[f] Laura Lega, MD,[g] Francesco Messina, MD,[h] Roberto Paludetto, MD,[b] and Roberto Berni Canani, MD, PhD[b,i]

[a]Department of Women's Health and Territorial Medicine, University La Sapienza, Rome, Italy; [b]Department of Pediatrics, University Federico II, Naples, Italy; [c]Neonatology Unit, Monaldi Hospital, Naples, Italy; [d]Department of Pediatrics, University La Sapienza, Rome, Italy; [e]Gastroenterology Unit, Cardarelli Hospital, Naples, Italy; [f]Neonatology Unit, Fatebenefratelli Hospital, Naples, Italy; [g]Neonatology Unit, Meyer Pediatric Hospital, Florence, Italy; [h]Neonatology Unit, V. Betania Evangelic Hospital, Naples, Italy; and [i]European Laboratory for the Investigation of Food Induced Diseases, Naples, Italy

Pediatrics, 2012, 129. e-40-45

Effect of H2 Blocker on Microbiome

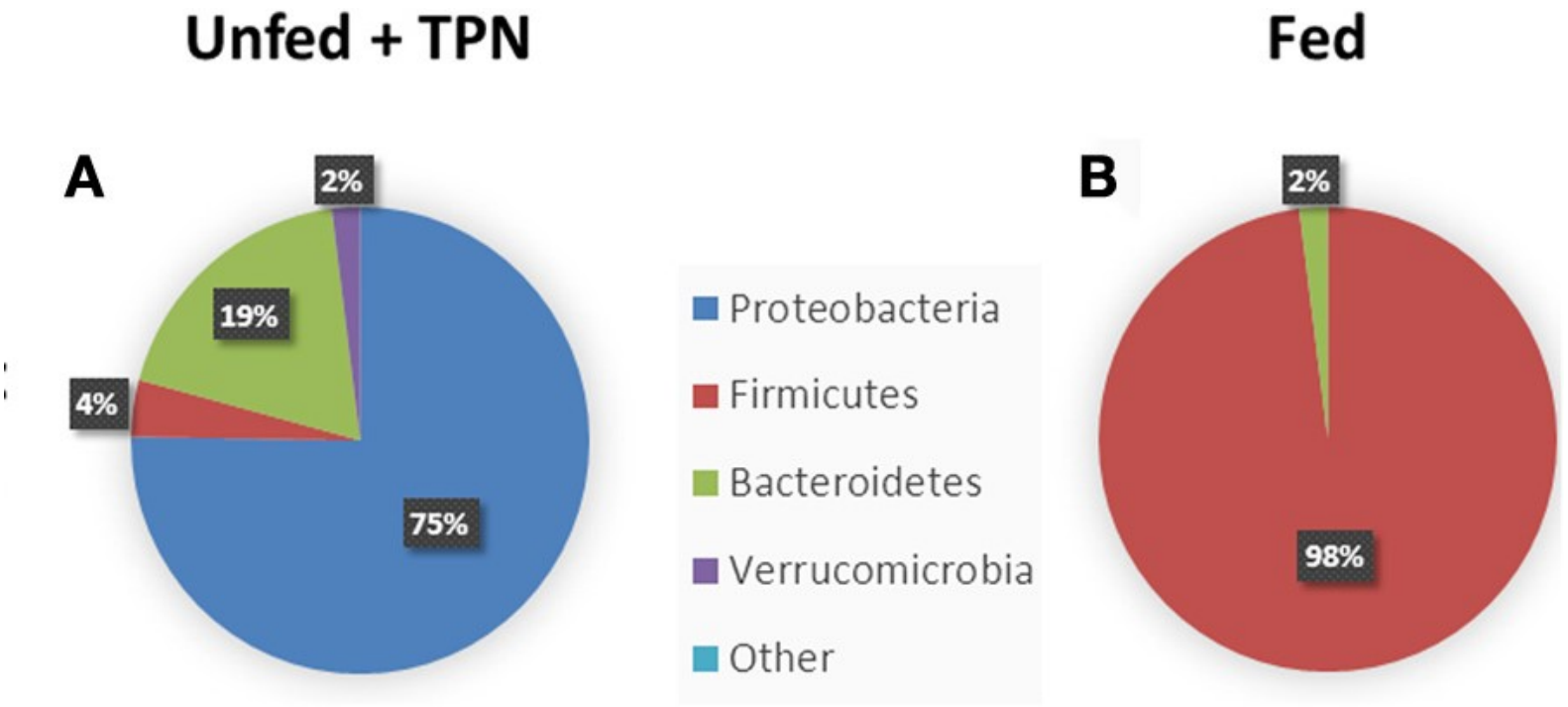


Gupta RW, et al., JPGN, 2013

Things we do to Mess Things Up: Lack of Enteral Feeding

- **Excuses To Withhold ENTERAL "Feedings**
  - ✓**Low APGAR scores.**
  - ✓**Umbilical catheters.**
  - ✓**Apnea and Bradycardia.**
  - ✓**Mechanical ventilation.**
  - ✓**CPAP.**
  - ✓**Vasoactive drugs.**
  - ✓**TPN is available.**

Effect of Total Parenteral Nutrition (TPN) in Mice



Demehri, FR., et al. Cellular and Infection Microbiology, Dec. 2013

Morbidities: Early vs. Late Feeding

Table 3. Univariate Analysis of Neonatal Morbidities by Group.

Outcomes (%)	Early (n = 79)	Late (n = 51)
NEC	6.3	10.0
ROP	16.7	52.1**
CLD	21.5	69.4**
PVL	0.0	6.0*
IVH	24.1	24.0
Comorbidities	8.0	25.0**

* Early vs. Late $p<0.05$;

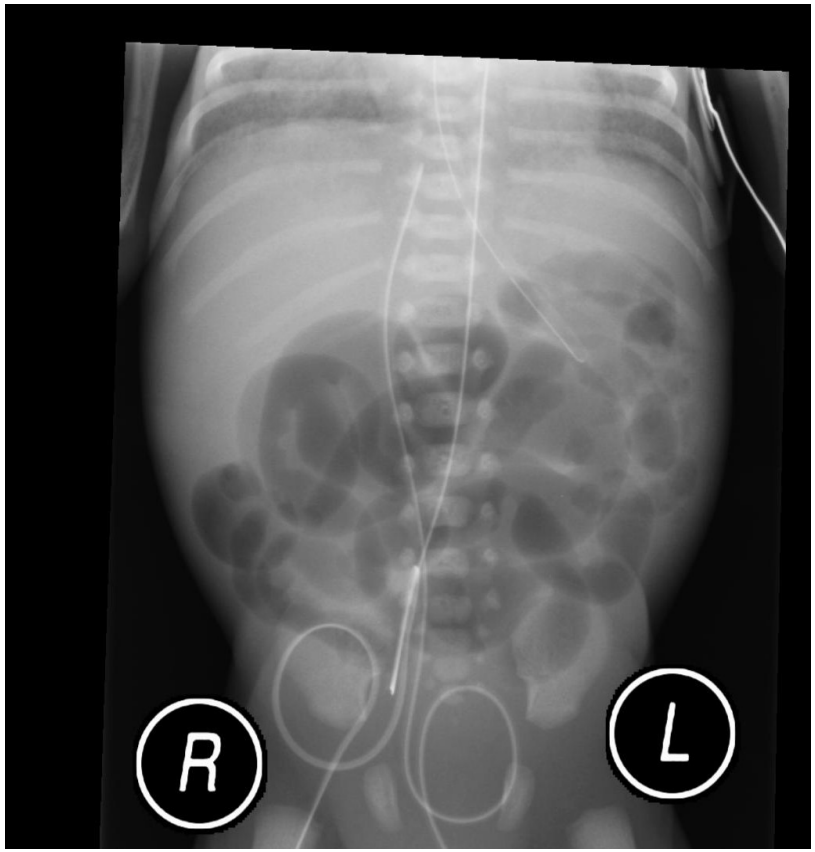
** Early vs. Late $p<0.0001$

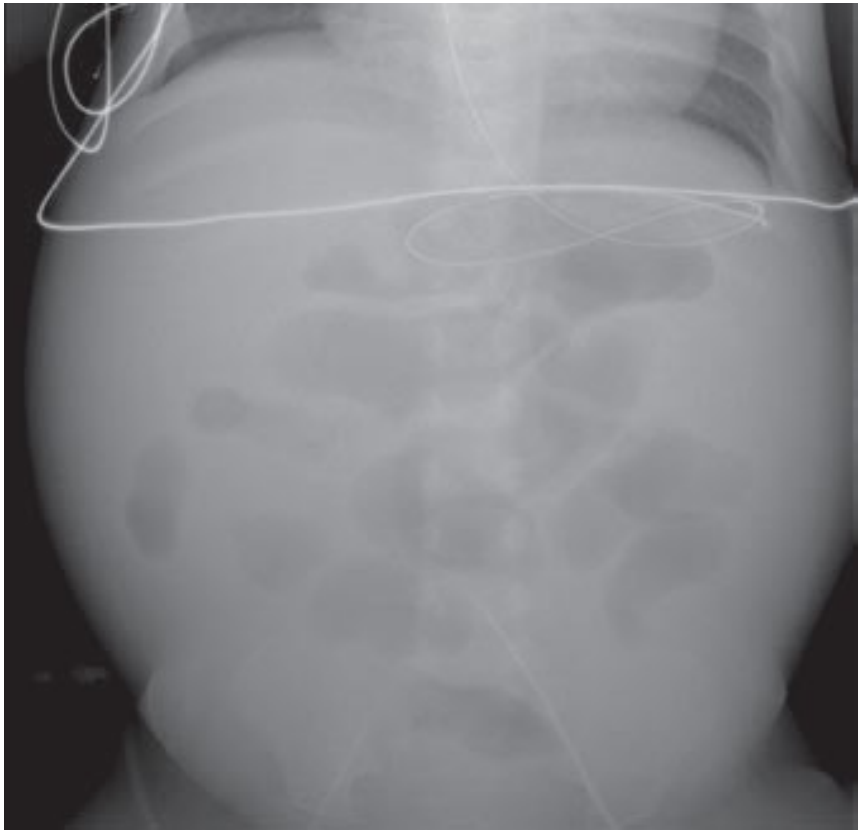
Necrotizing Enterocolitis (NEC); Retinopathy of Prematurity (ROP); Chronic Lung Disease (CLD); Periventricular Leukomalacia (PVL); Intraventricular Hemorrhage (IVH); Cormorbidities = The presence of 2 or more neonatal outcomes.

Konnikova, et al. PLOS One 2015


The Future
NEXT EXIT

NEC: A Diagnostic Dilemma





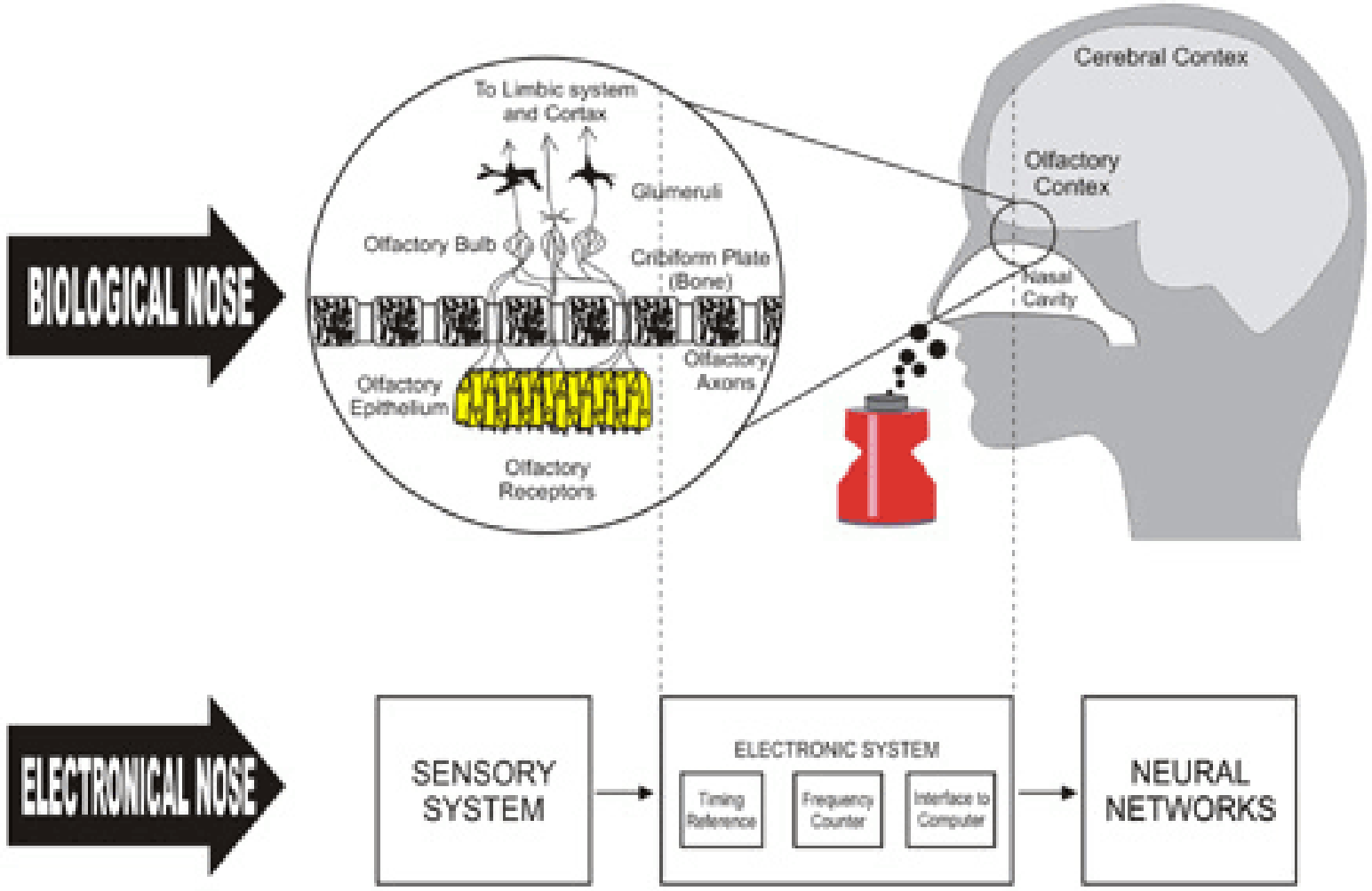
NEC versus Non NEC Differentiation

Thuijls, et al. Annals of Surgery, 251 (6), June 2010

Marker	Cutoff Point	Sensitivity	Specificity	LR+	LR-	AUC (95%CI)	P
I-FABP	2.25 pg/mmole creatinine	0.93	0.90	9.3	0.08	0.98 (0.94-1.0)	<0.001
Claudin-3	8OO.8 INT	0.71	0.81	3.74	0.36	0.76 (0.59-0.94)	0.016
Calprotectin	286.2 microgram/gram feces	0.86	0.93	12.29	0.15	0.94 (0.85-1.0)	0.001

ELECTRONIC NOSE CONCEPT

4to40

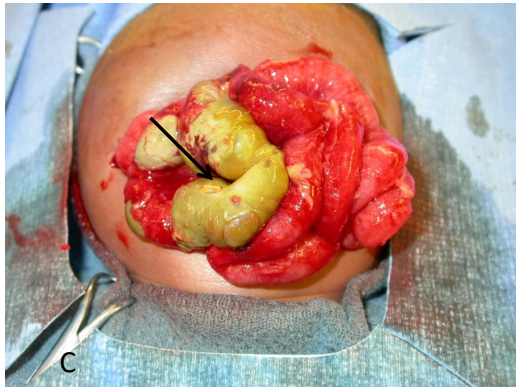


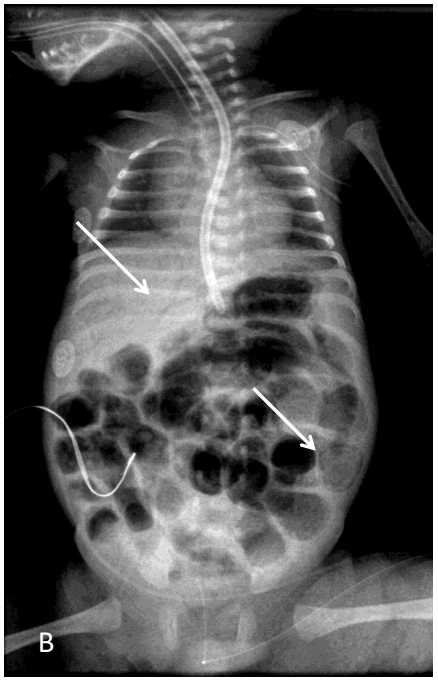
Ea anic

Tim (de, MD[1],
Anna E. MD, PhD[5],
nd

Objec onic nose
(eNose
Study eeks were
collect matched
control d 2 days
before Nose.
Resul d controls
(n = 14 %, 75%);
the ac were also
signific ut not at
$T_{-1,0}$ ((
Conc could be
discrim gest that
VOC-profiling by eNose has potential as a noninvasive tool for the early prediction of NEC. *(J Pediatr 2015;167:562-7).*

“Classic” NEC: Probiotics





Neu, J. and Walker , W. A. New England Journal of Medicine, Jan. 2011

Routine Use of Probiotics

Meta-Analysis -NEC

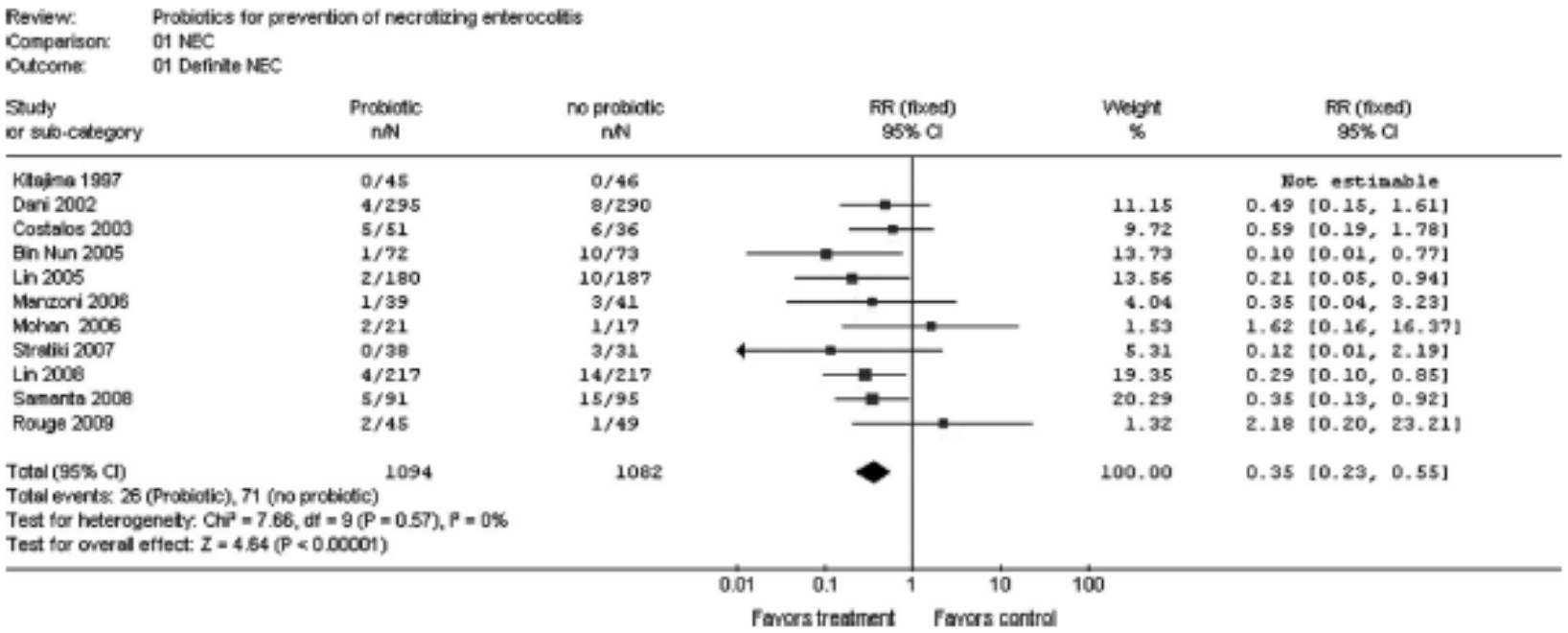


FIGURE 2
Effect of probiotics on NEC.

Deshpande, G. Pediatrics, 2010

Summary of 2010 Meta Analysis

- 11 studies evaluated.
- 10 different probiotic preparations.
- Risk for NEC and death significantly lower in probiotic group.
- Sepsis did not differ.
- “Overall evidence indicate that additional placebo controlled trials are unnecessary if a suitable probiotic product is available”.

COMMENTARY www.jpeds.com • THE JOURNAL OF PEDIATRICS

Routine Probiotics for Premature Infants: Let's Be Careful!

Josef Neu, MD

Recent technological advances have provided us with the knowledge that when one evaluates the number of genes or cells in the average human being, only 10% are mammalian, and the rest are microbial.[1,2] The recent development of non-culture-based techniques to

to such a parental request, should the physician provide the infant with whatever probiotic product the parent wishes to provide to the infant? Is there a "suitable probiotic product" with adequate quality control that is approved by the Food and Drug Administration (FDA) (as safe and effective

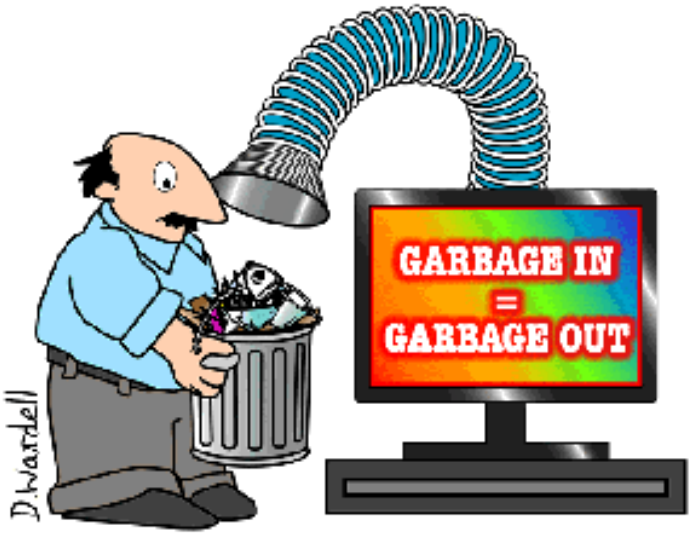
J Pediatr. 2011 Apr;158(4):672-4.

Systematic Reviews: The Good, the Bad, and the Ugly

Yuhong Yuan, MD, PhD[1] and Richard H. Hunt, MB, FRCP, FRCPC, FACG, AGAF[1]

Systematic reviews systematically evaluate and summarize current knowledge and have many advantages over narrative reviews. Meta-analyses provide a more reliable and enhanced precision of effect estimate than do individual studies. Systematic reviews are invaluable for defining the methods used in subsequent studies, but, as retrospective research projects, they are subject to bias. Rigorous research methods are essential, and the quality depends on the extent to which scientific review methods are used. Systematic reviews can be misleading, unhelpful, or even harmful when data are inappropriately handled; meta-analyses can be misused when the difference between a patient seen in the clinic and those included in the meta-analysis is not considered. Furthermore, systematic reviews cannot answer all clinically relevant questions, and their conclusions may be difficult to incorporate into practice. They should be reviewed on an ongoing basis. As clinicians, we need proper methodological training to perform good systematic reviews and must ask the appropriate questions before we can properly interpret such a review and apply its conclusions to our patients. This paper aims to assist in the reading of a systematic review.

Am J Gastroenterol 2009;104:1086–1092; doi:10.1038/ajg.2009.118



Mistake: Pooling of data across trials as if they belonged to a single large trial.

Food Supplement or Drug?

- It depends!
- Medical claim (e.g. prevention of NEC, treatment of diarrhea) usually should be considered a drug.
- Drugs that are sold by prescription are subjected to rigorous testing.
- Foods can be sold by anyone and not subjected to rigorous standards.

FDA ALERT!!

Fatal gastrointestinal mucormycosis in an infant following use of contaminated ABC Dophilus from Solgar Company

CDC, FDA, and the Connecticut Departments of Public Health and Consumer Protection, are investigating a fatal case of GI mucormycosis in a premature infant of 29 weeks gestation following the use of a probiotic supplement called ABC Dophilus distributed by Solgar, Inc., Leonia, NJ.

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6406a6.htm

Largest Study so Far: UK Pips Trial (Costello, et al. Lancet Feb., 2016)

- Double Blinded, randomized, Prospective.
- Bifidobacterium breve probiotic
- 23-31 weeks gestational age enrolled
- Powered for NEC as primary outcome: 1315 infants enrolled
- Results: NO differences in:
  - NEC
  - Late Onset Sepsis
  - Death

Summary and the Future

- NEC Pathogenesis is Multifactorial (Even if we invoke a “Classic NEC”). We need better definitions.
- Treatment of NEC once it is developed is extremely difficult. We need to prevent.
- The intestinal microbial environment along with developmental aspects of the GI tract are key in understanding the pathogenesis of NEC.
- We need to have better systems (enteroids, animal models) to evaluate mechanisms that fulfill criteria for causality derived from strong associations found in humans.
- Once we have clear understanding of the causes of the different forms of NEC, we will be best able to target preventative strategies.