Figure 1 | Home for an abundant microbiological flora. The human gut and (inset) a scanning electron micrograph of part of the small intestine, with some bacterial inhabitants picked out in green.

Probiotics in Functional Bowel Disorders: Principles and Evidence

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Topics

- Probiotics and public perceptions
- The gut microbiome and its development
- Rationale for probiotics in IBS-FBD
- Animal studies
- Human microbiome studies
- Clinical studies with probiotics for IBS
- Putting it together: meta-analysis and Syst Review
- Safety concerns?

Definitions

- Probiotics

Live microorganisms which when administered in adequate amounts confer a health benefit on the host

Food and Agriculture Administration/WHO (2001)

- Prebiotics

Non-digestible food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract

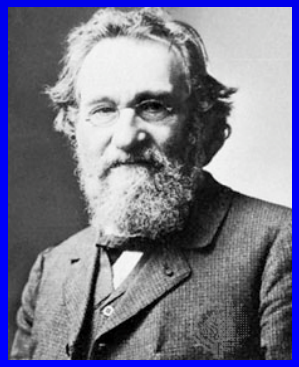
  - Inulin
  - Fructose oligosaccharides
  - Oligosaccharides in human breast milk

Selectively stimulates the growth and activity of certain species of intestinal bacteria

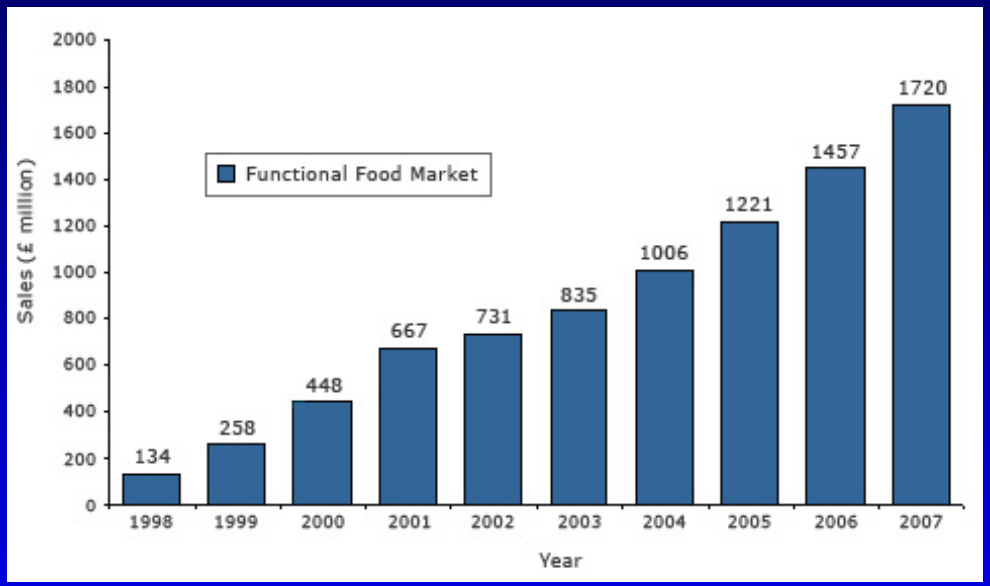
  - *Bifidobacteria*
  - *Lactobacilli*

- Synbiotics

Prebiotics + Probiotics



Interest and spending in functional foods is climbing steadily



- Seen as natural alternative to traditional medical Rx
- Direct to consumer advertising
- Claims often not justified by evidence
- Claims not generally evaluated by FDA
- "This product is not intended to diagnose, treat, cure, or prevent any disease"

"Functional foods" = foods or dietary components that may provide a health benefit beyond basic nutrition

Sample claims

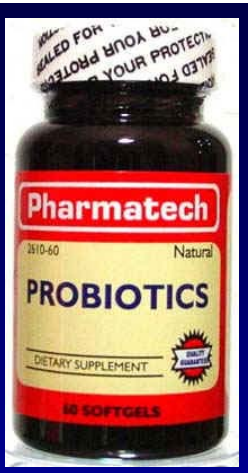
"promotes a healthy digestive system to help restore your natural balance (*B. infantis*)

"Once daily capsule contains probiotics to help with occasional constipation, diarrhea, gas, and bloating"

"…helps to naturally regulate your intestinal transit"

"Reduces bloating, gas and intestinal discomfort. Supports regularity"

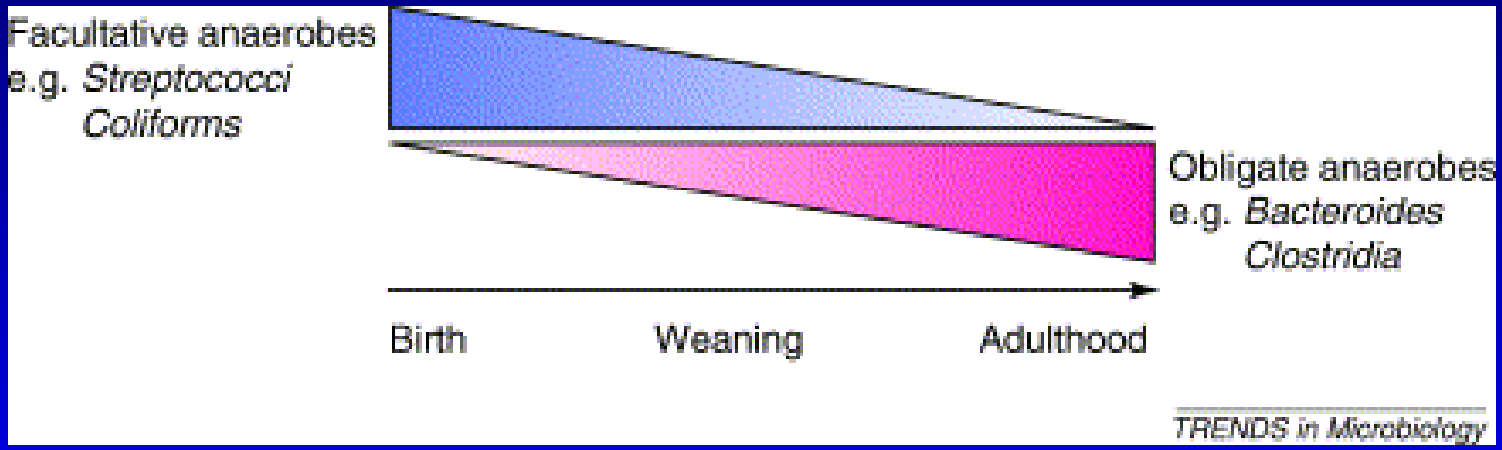
False advertising



- 5 human and 8 veterinary probiotics examined
- Only 2 matched contents on label.
- Many bacteria with no known probiotic properties
- Several putative pathogens

Weese ‘02

Gut flora composition changes during life



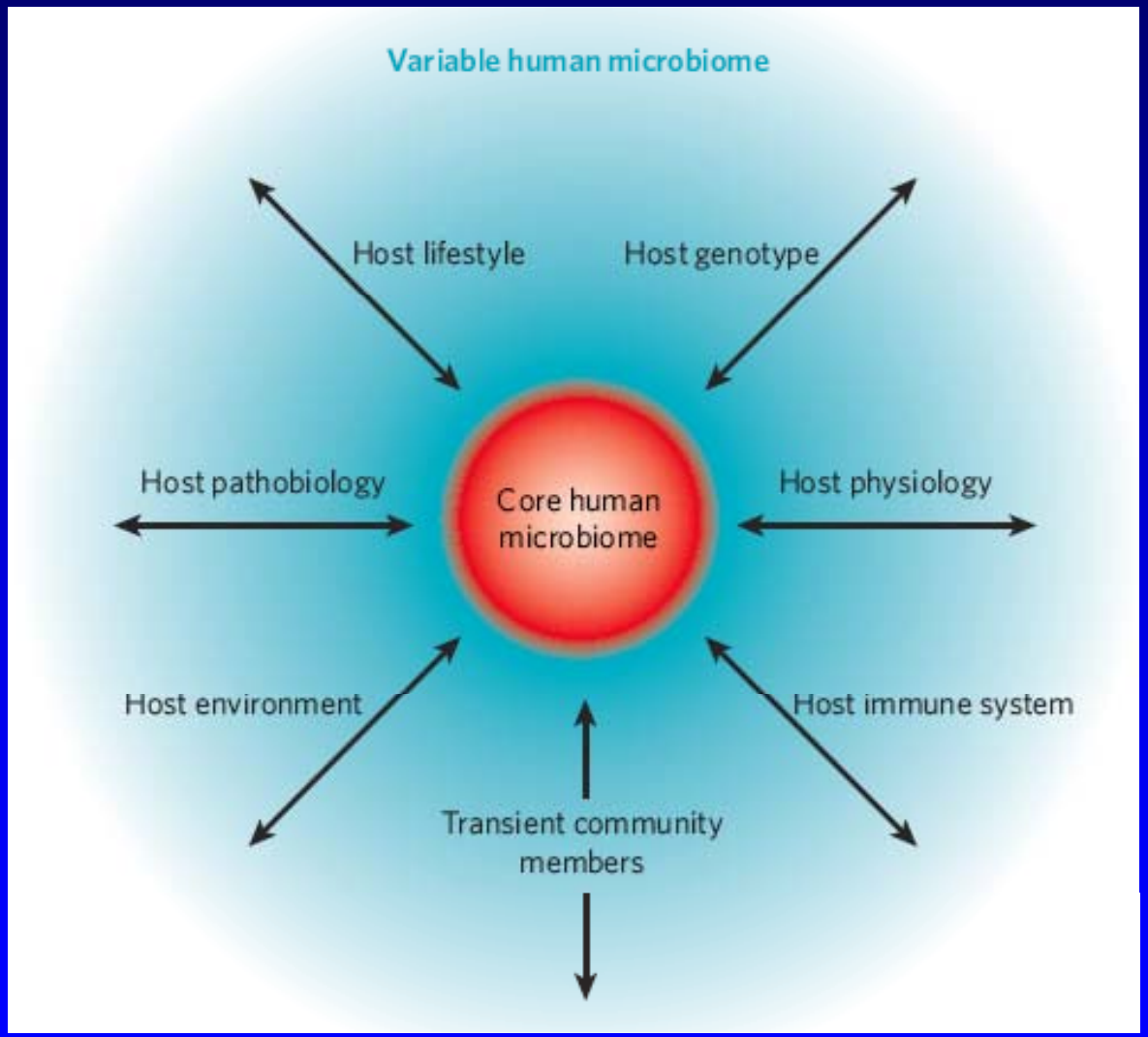
Factors:

Age
Diet
Health status

Implications

- Microbial colonization of neonate NOT STEREOTYPICAL.
  - Random and chaotic
  - Initial composition may depend on exposures
  - Maternal “signatures” from breast milk, vagina--> not persisting
- Environmental and incidental exposures likely determine early colonization.
- “Well-adapted” adult flora eventually outcompete the other colonizers.

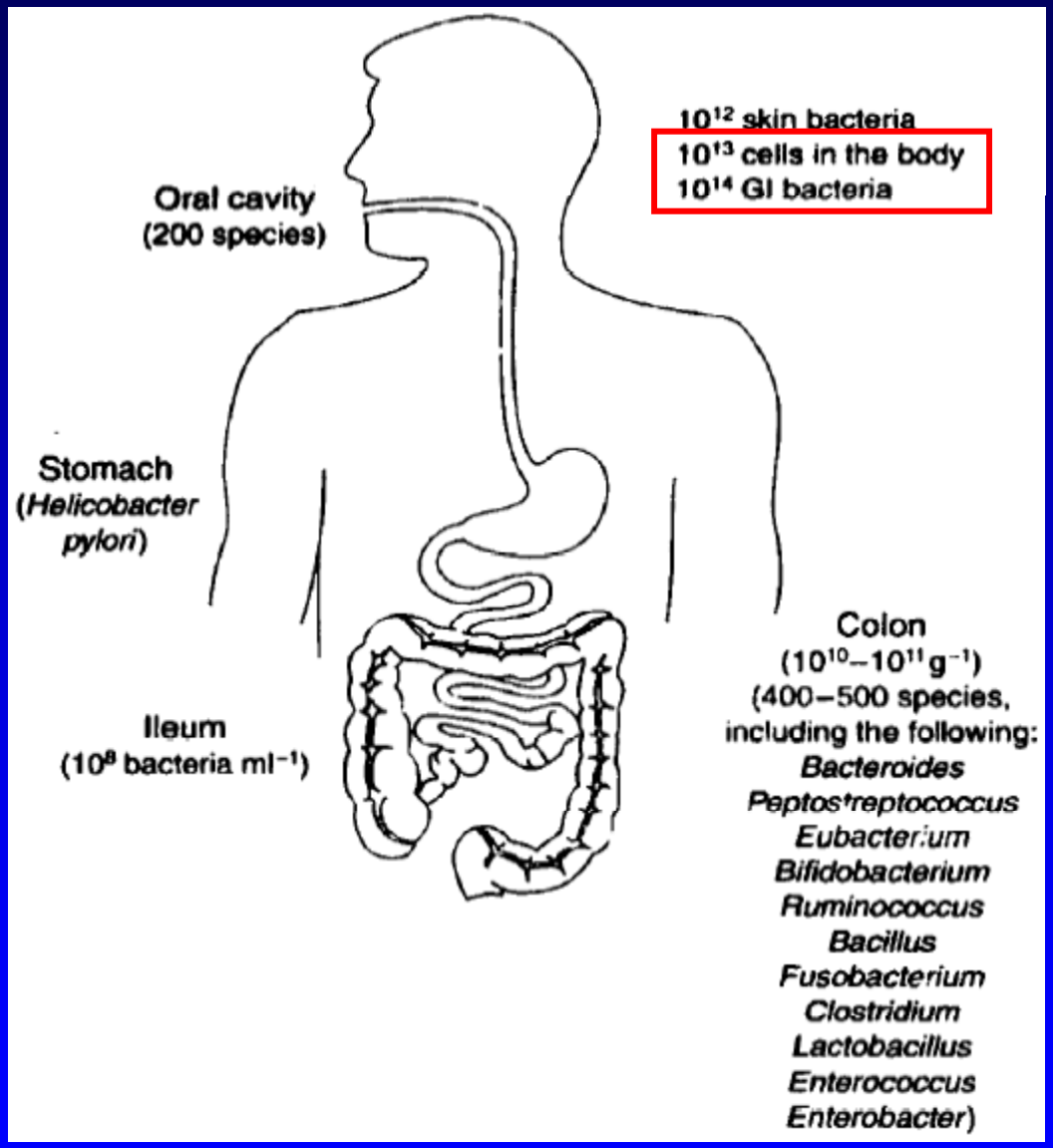
Common and variable components make up the gut microbiome



Humans 1 Bacteria 10

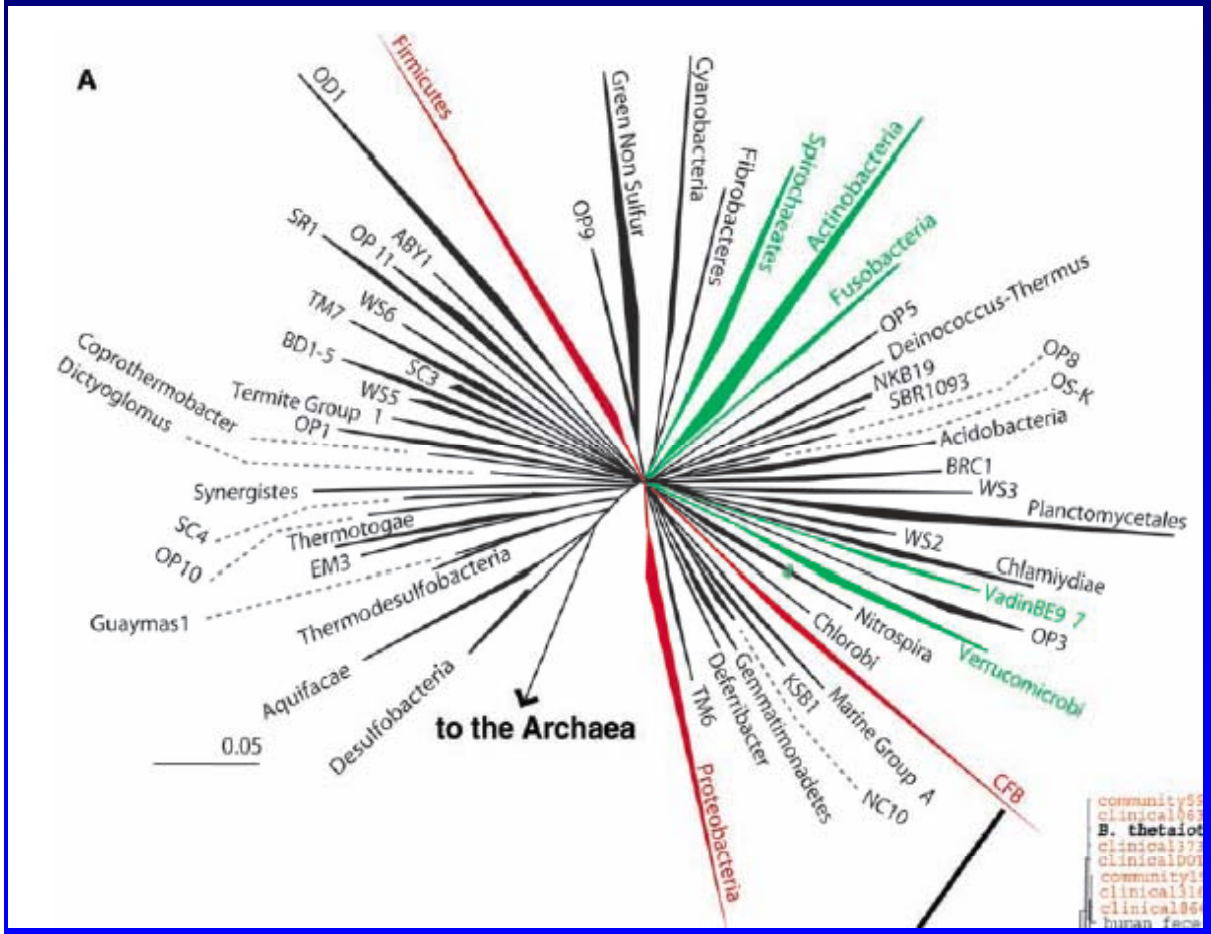
Saliva: 10^9 /ml
Dental plaque:
10^{11} /gm

Upper GI:
10^{3-4} /ml contents



99.9% obligate anaerobes

Diversity of bacteria in the human gut



- Based on sequencing of ~9000 16S rRNA genes
- Each wedge represents a “division” or superkingdom
  - Abundant
  - Rare
  - Undetected
- >90% of bacteria belong to only 2 divisions out of ~70
  - Firmicutes
  - Bacteriodetes

Is the gut microbiota important in IBS pathogenesis?

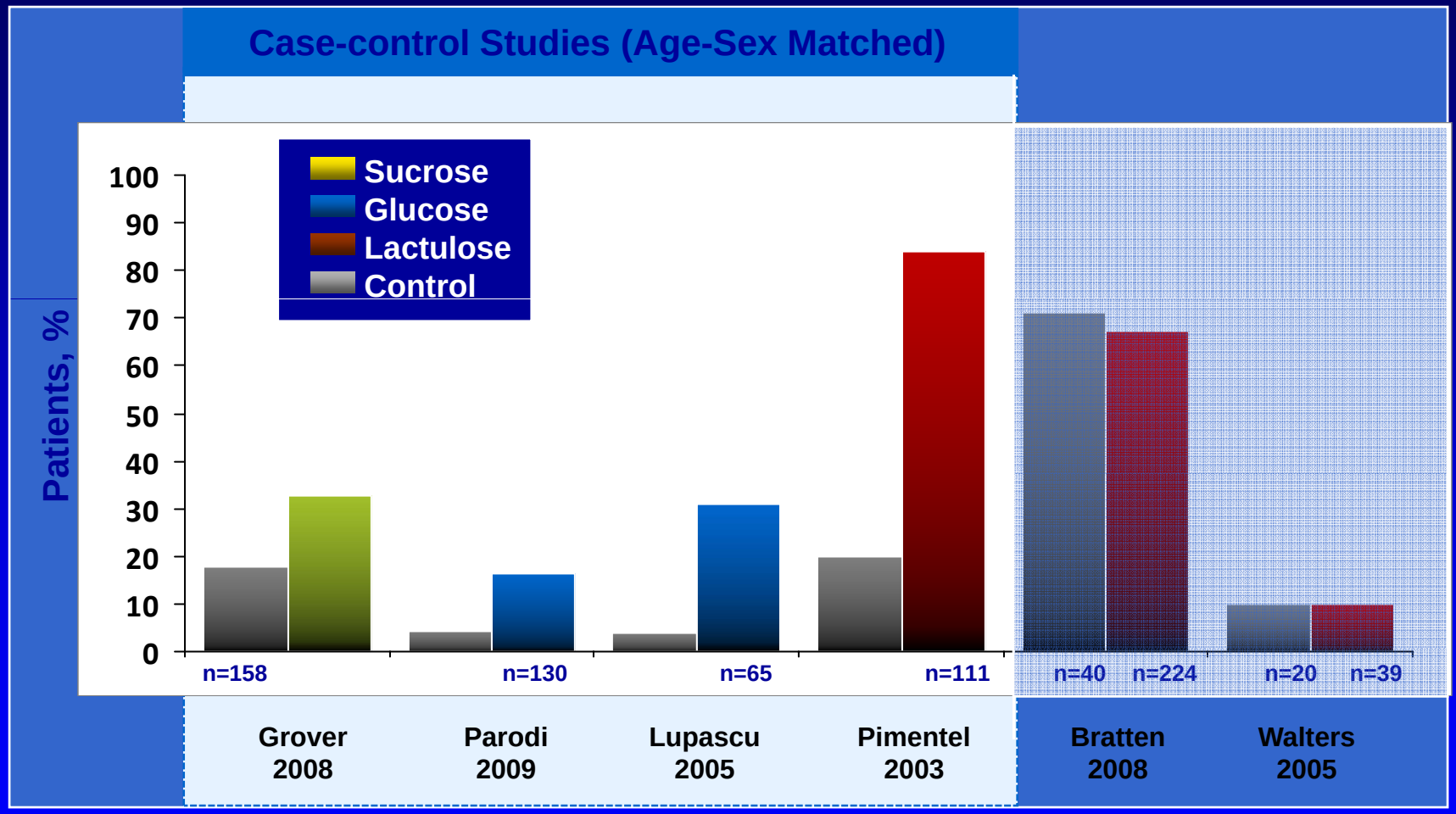
- Differences in the fecal and mucosa-associated microbiota (MAM) of IBS pts and healthy controls
- Increasing evidence of activation of intestinal immune system in IBS
- Infectious trigger in PI-IBS
- Link between SIBO and IBS

PI-IBS after acute gastroenteritis

Author	Follow Up	PI-IBS	%
McKendrick et al 1994	12m	12/38	31
Gwee et al 1999	3m	22/100	22
Neal et al, 1997 & 2003	6m 6yr	23/357 14/192	7 7.3
Thornley et al 2000	6m	9/93	9
Okhuysen, et al 2004	6m	6/60	10
Mearin, et al, 2005	12m	24/271	10
Marshall, et al, 2005	2-3 yrs	380/1137	33.5%

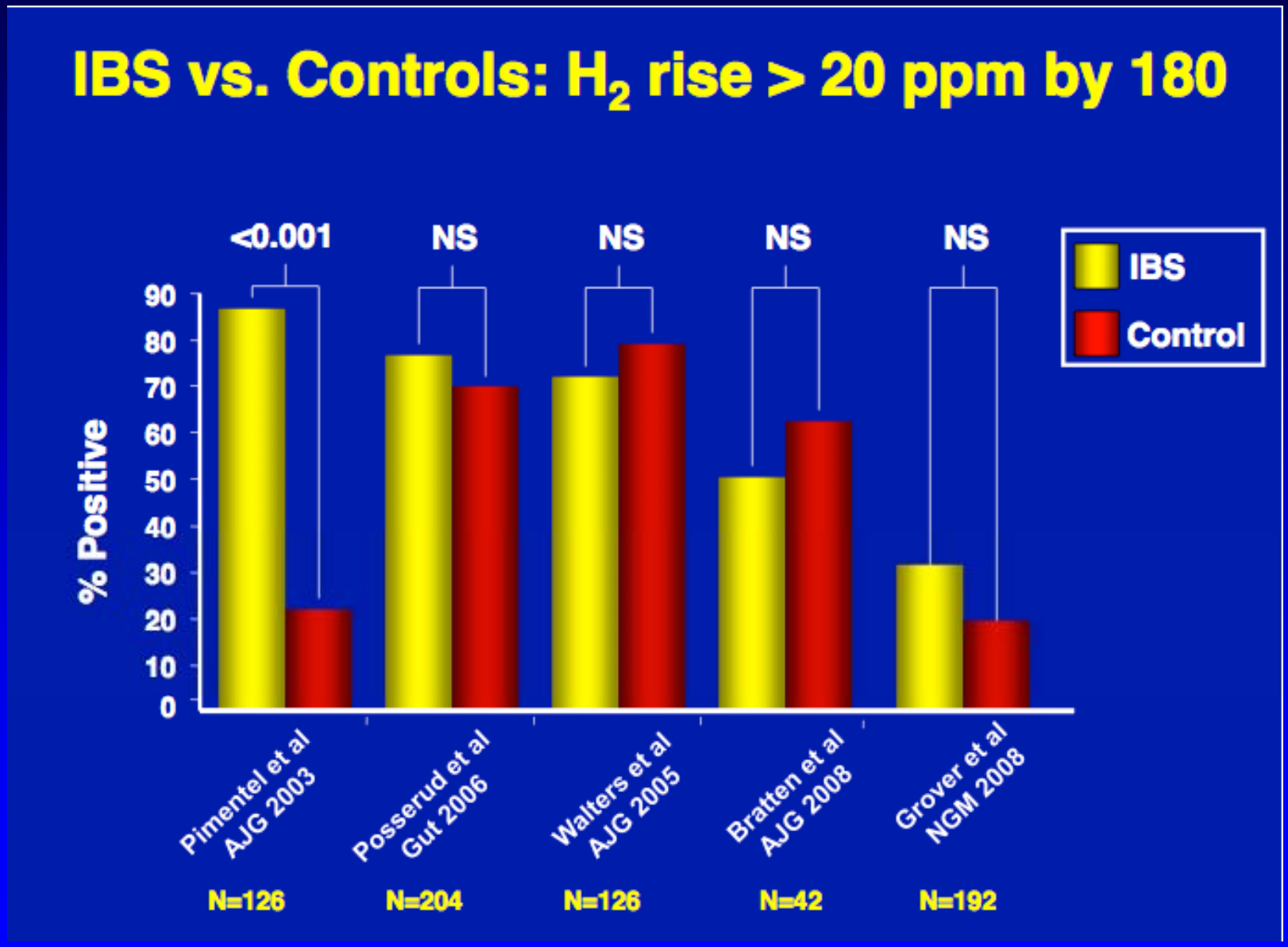
Halvorson ‘06 PI-IBS meta-analysis: 9.8% prevalence in Infectious diarrhea group. 1.2% in controls

Breath testing and IBS: Point



Pimentel 2009

Breath testing and IBS: Counterpoint



Spiegel, post DDW 2009

- A number of studies show high prevalence of SIBO in IBS patients, based on HBT
- Antibiotics can improve IBS symptoms in a significant proportion of these patients

Germ-free rodents have altered motility

Neuromuscular / Motor Event	Finding	Bacteria
Enteric nervous system	Reduced GABA and VAP-33 gene expression	*Bacteroides thetaiotaomicron*
Intestinal smooth muscle	Reduced γ-actin and CRP-2 gene expression	*Bacteroides thetaiotaomicron*
Gastric emptying	Delayed	Whole flora
Intestinal transit	Delayed	Whole flora; *Lactobacillus acidophilus* and *Bifidobacterium bifidum*
MMC cyclic recurrence and distal propagation	Reduced	Whole flora
Cecal size	Enlarged	Whole flora; anaerobic flora

GABA, gamma-amino butyric acid; VAP, vesicle-associated protein; CRP, cysteine-rich protein; MMC, migrating motor complexes.

Functions normalized following introduction of normal intestinal flora

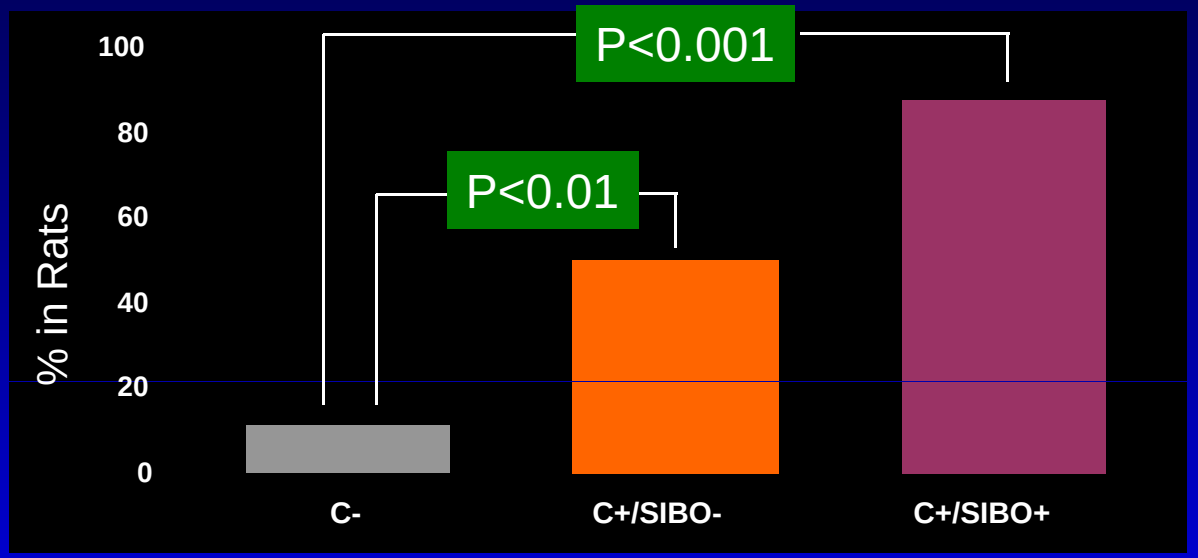
Implications:

- Enteric microflora play an important role in maintaining normal intestinal function
- Changes in intestinal microflora can lead to significant alterations in GI function

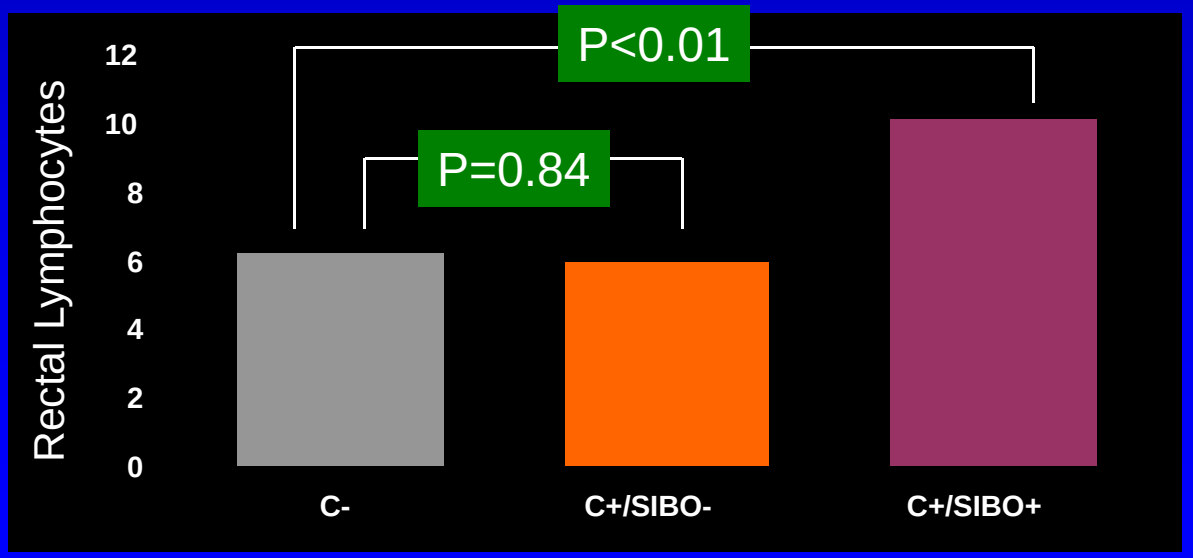
Barbara, AJG '05

PI-IBS rat model 3 months post-*Campylobacter jejuni* infection

Persistent altered stool consistency



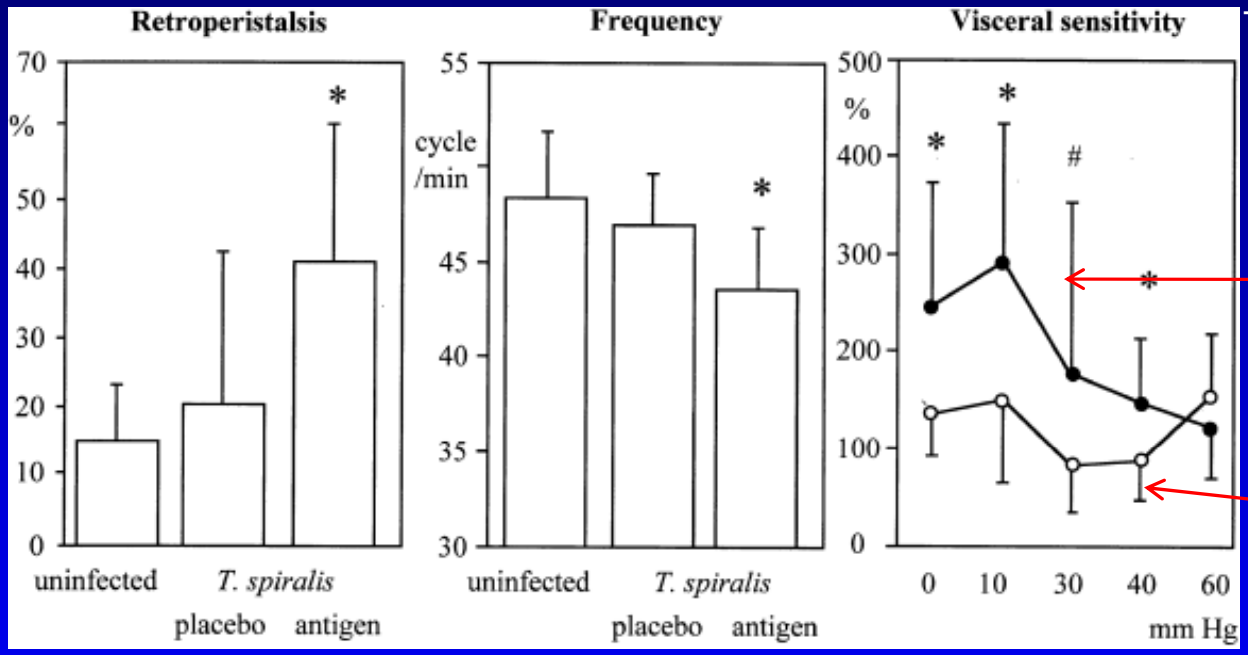
Increased rectal lymphocytes



Pimentel, et al. '08 Dig Dis Sci 53:982

Alterations in gut motility and hyperalgesia in *Trichinella spiralis* mouse infection model

Day 70
Post infec



Probiotic effects on murine pain receptors

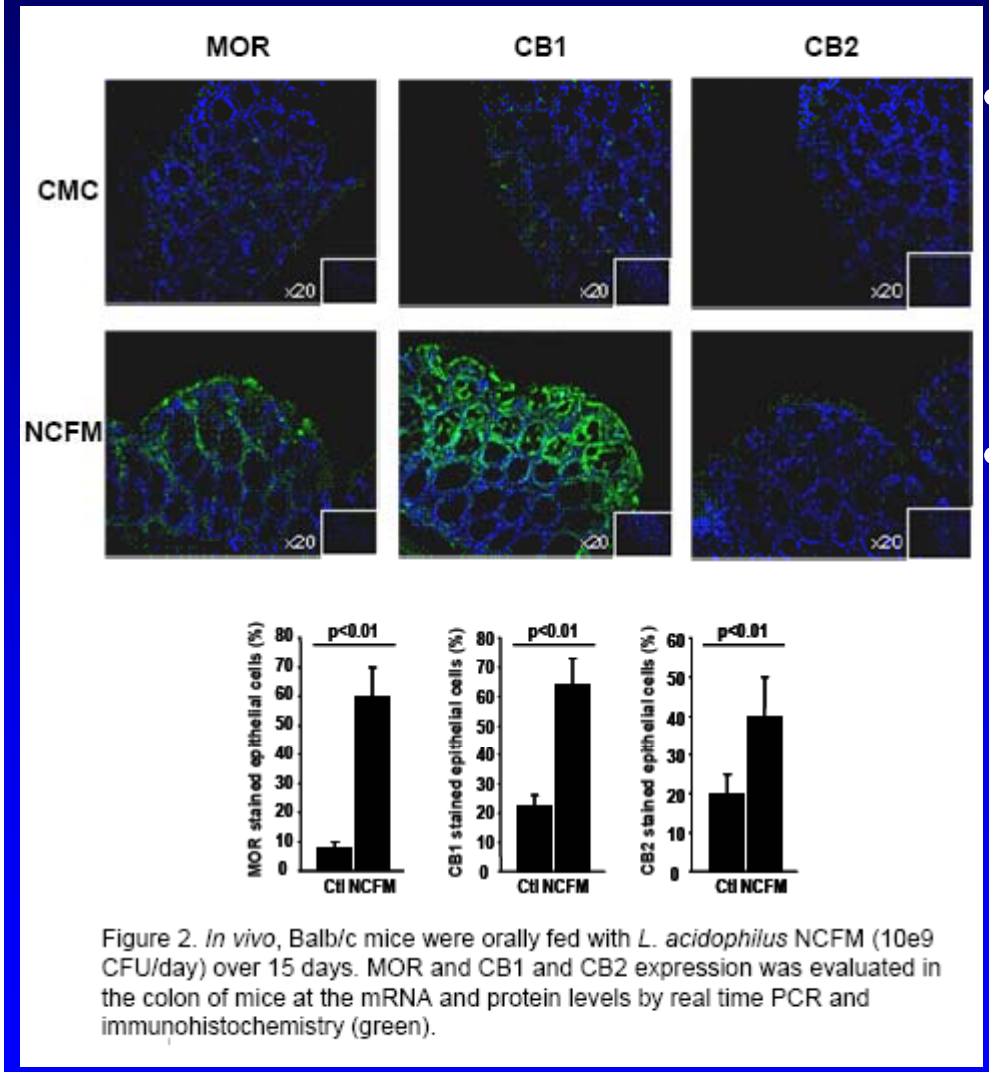
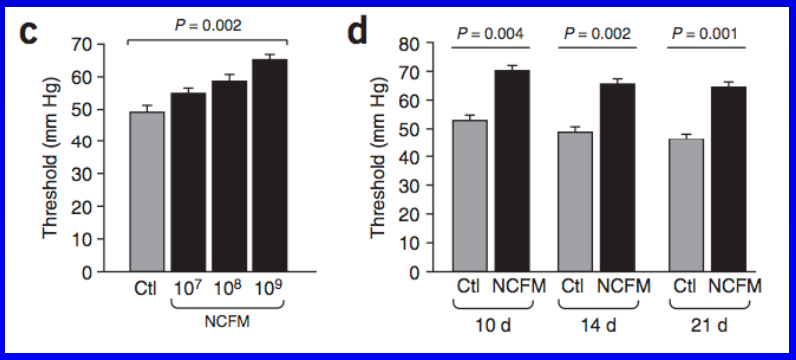


Figure 2. *In vivo*, Balb/c mice were orally fed with *L. acidophilus* NCFM (10e9 CFU/day) over 15 days. MOR and CB1 and CB2 expression was evaluated in the colon of mice at the mRNA and protein levels by real time PCR and immunohistochemistry (green).

- *L. acidophilus* NCFM induces expression of intestinal pain receptors (MOR, CB1, CB2)
- Decreased visceral sensitivity



Rousseaux, Nature Med '07

Limitations in evaluating gut microbiota in IBS

- Complete composition of GI microbiota remains uncharacterized
- Heterogeneity of functional bowel disorders
- Stability of intestinal microbiota in IBS patients may fluctuate
- Topographic/geographic variability in the GI microbiota
- Mucosa-associated microbiota may differ from lumenal bugs
- Other factors may affect gut microbiota: motility, diet, meds
- Current methodologies to assess the densities and composition of gut microbiota have inherent limitations

Ringel, GECNA'09

Altered microbiota in colon of IBS patients

Table 1 Results of recent studies on the composition of the fecal microbiota in irritable bowel syndrome patients

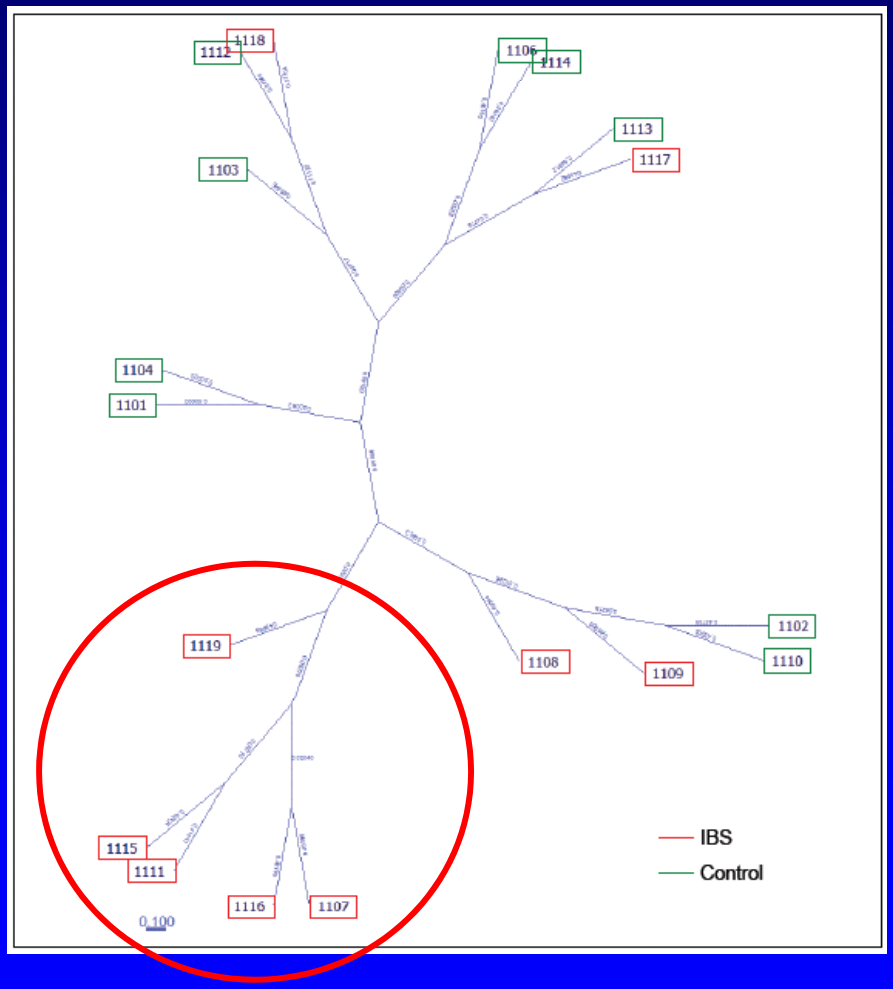
Reference	No. IBS patients	Method	Outcome
Si *et al.*[19] (2004)	Unsubtyped IBS (n = 25)	Culture	↓*Bifidobacteria* and ↑*Enterobacteriaceae* in IBS patients
Matto *et al.*[20] (2005)	IBS-D (n = 12) IBS-C (n = 9) IBS-M (n = 5)	Culture PCR-DGGE	↑Coliforms and ↑aerobic bacteria/total bacteria in IBS patients ↑*Clostridium* and ↓*Eubacterium* in IBS patients
Malinen *et al.*[22] (2005)	IBS-D (n = 12) IBS-C (n = 9) IBS-M (n = 6)	qPCR	↓*Lactobacillus* in IBS-D patients and ↑*Veillonella* in IBS-C patients
Maukonen *et al.*[23] (2006)	IBS-D (n = 7) IBS-C (n = 6) IBS-M (n = 3)	PCR-DGGE RT-PCR-DGGE	↓*Clostridium coccoides-Eubacterium rectale* in IBS-C patients
Kassinen *et al.*[24] (2007)	IBS-D (n = 10) IBS-C (n = 8) IBS-M (n = 6)	qPCR with nucleic acid fractionation	↓*Collinsella*, *Clostridium*, and *Coprococcus* species in IBS patients
Kerckhoffs *et al.*[25] (2009)	IBS-D (n = 14) IBS-C (n = 11) IBS-M (n = 16)	FISH	↓*Bifidobacterium* in IBS patients
Tana *et al.*[4] (2010)	IBS-D (n = 8) IBS-C (n = 11) IBS-M (n = 7)	Culture qPCR	↑*Lactobacillus* in IBS patients ↑*Veillonella* in IBS patients

DGGE, denaturing gradient gel electrophoresis; FISH, fluorescent in situ hybridization; IBS, irritable bowel syndrome; qPCR, quantitative real-time polymerase chain reaction.

- Inconsistent results on the species found in feces of IBS
  - Disease heterogeneity
  - Different techniques and resolving power
  - Feces composition does not represent entire gut microbiota

Lee, NGM ‘10

The gut microbiota in D-IBS differs from healthy controls



- T-RFLP – a non-selective method to investigate complex microbial community using 16SDNA genetic bacterial profiles
- Genetic profiles generated from fecal samples of D-IBS and controls
- Significant portion of the
- fingerprint profiles from D-IBS clustered separately from controls

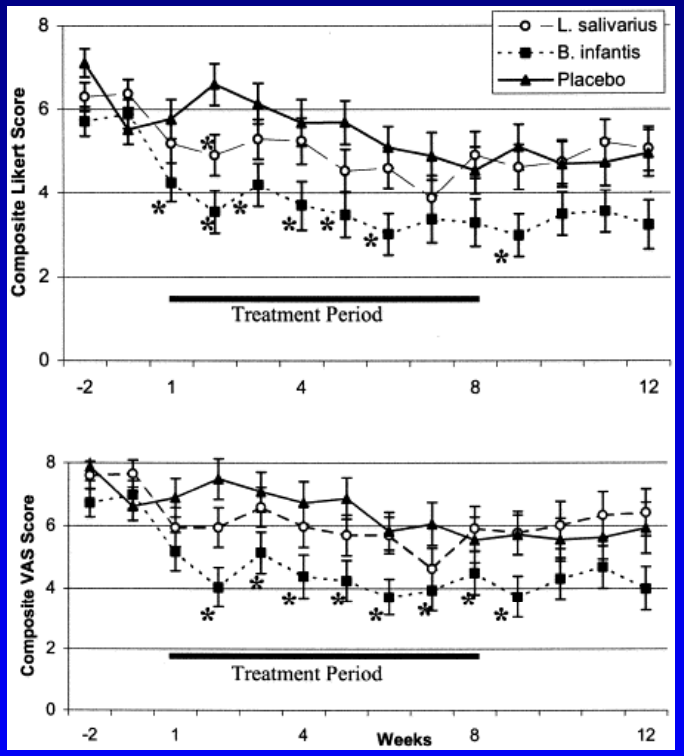
Carroll, NGM ‘09

Potential mechanisms of probiotic effects on functional bowel disease

- Interaction with host GI mucosal immune system
- Direct modulation of intestinal pain
  - Upregulation of Mu and cannaboid receptors by *L. acidohilus*
- Alteration of GI mucosal integrity
  - Induction of mucin gene MUC2 expression in colon by VSL#3
- Reduction of bacteria-produced intracolonic gas
  - Increased *Lactobacilli and Bifidobacteria*
  - Decreased *Clostridia and Veillonella*
- Increased production of intracolonic SCFA and increased colonic propulsion
- Reduced bile acid malabsorption in D-IBS

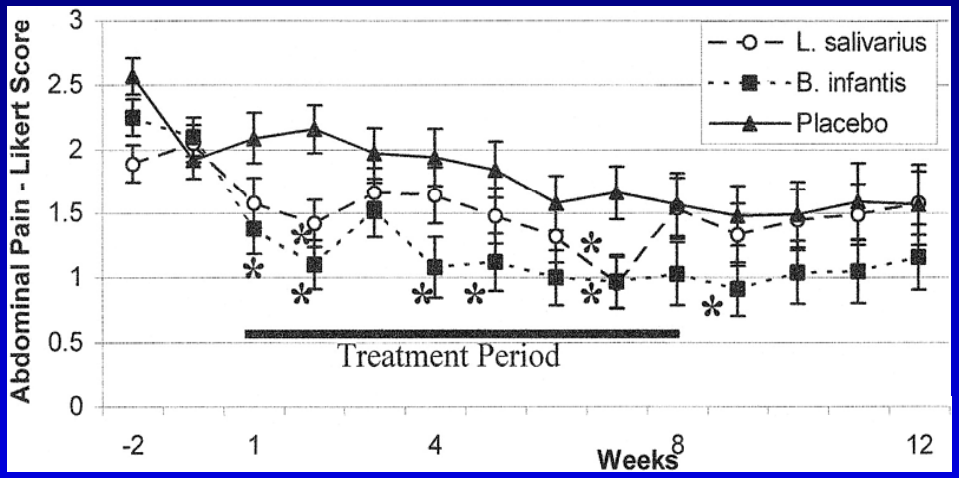
Bifidobacterium infantis and *Lactobacillus salivarius*

Composite of IBS symptoms
(Likert scale and VAS scores)



Significant reduction in composite scores

Abdominal pain scores
(Likert scale)



Significant improvement in pain/discomfort

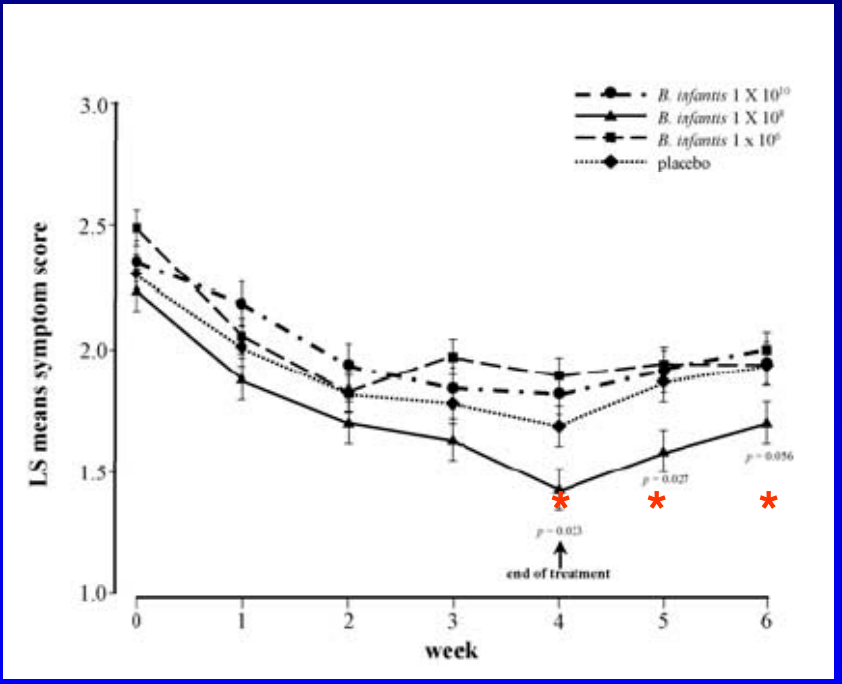
The beneficial effects were with *B. infantis* but not with *L. salivarius* or placebo (**P* < .05)

O'Mahony '05, Gastro

B. infantis

- Large-scale, multicenter, clinical trial
- 362 women with IBS
- Primary care clinics
- Placebo vs *B. infantis* at dose of
- 1 x 10^6 CFU/ml
- 1 x 10^8 CFU/ml
- 1 x 10^{10} CFU/ml
- 4 week treatment and 2 week washout

Abdominal pain scores
(Likert 0 to 5 scale)

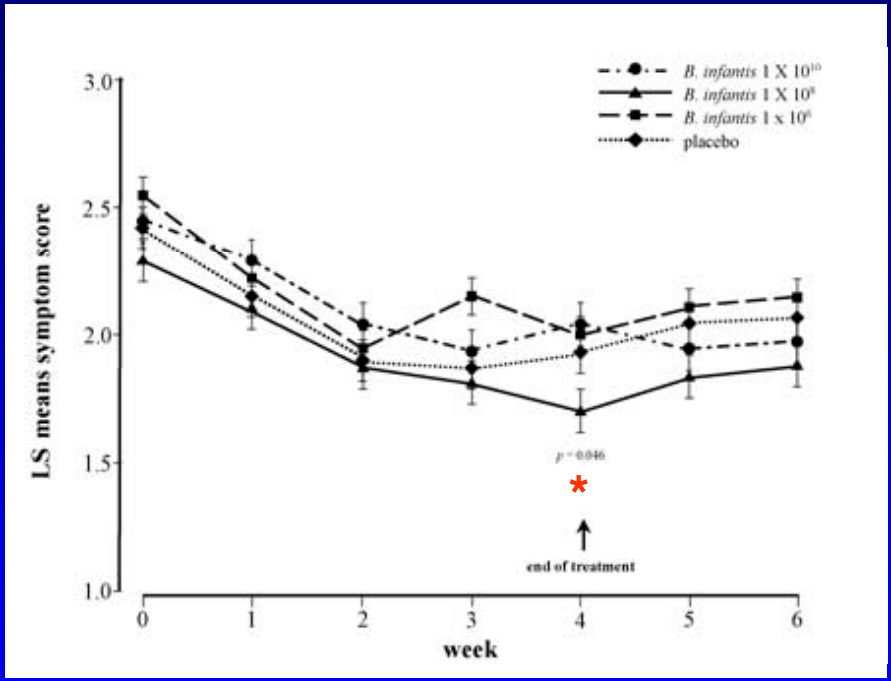


Improvement in pain/discomfort score at end of treatment (4 weeks) with *B. infantis 35624* 1 × 10^8 (*$P < .05$)

Whorwell '06 AJG

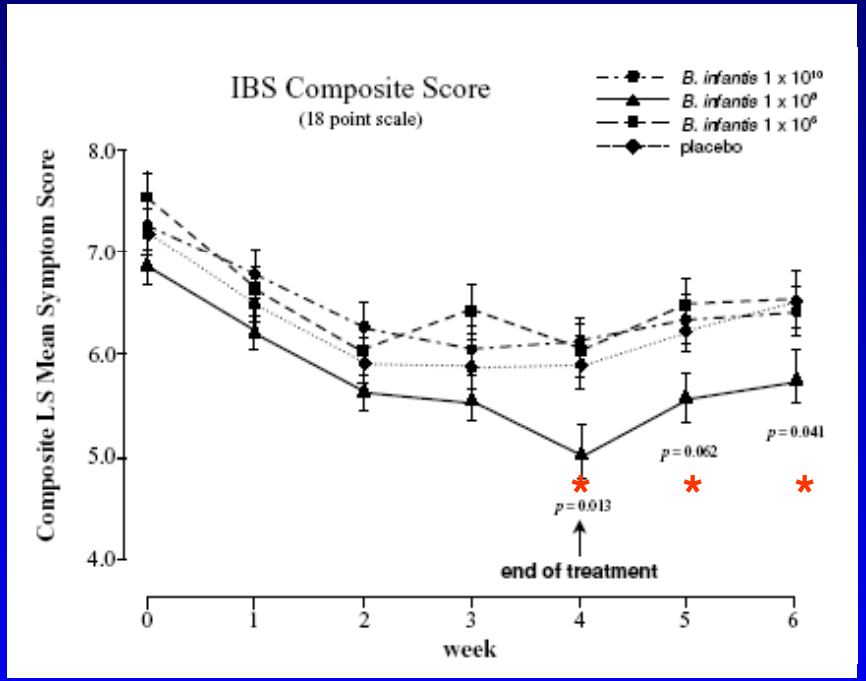
B. infantis

Bloating/Distention
(Likert 0 to 5 scale)



(*P < .05)

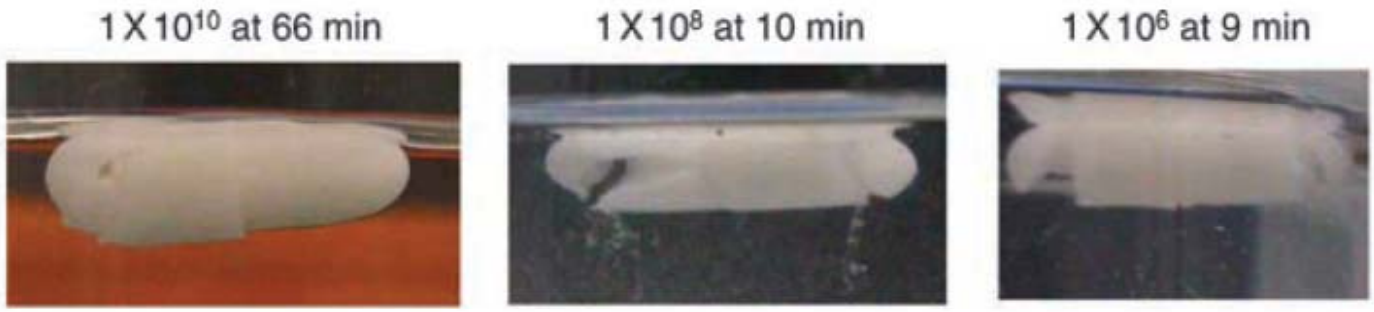
IBS Composite Score
(Likert 0 to 15 scale)



(*P < .05)

- All clinical benefits limited to 1x10^8 dose !?

Slow, inefficient capsule rupture



Capsule contents more solidified

L. plantarum 299v

- Complete pain resolution at 4 wks ($p=0.0012$)
- Improved global IBS symptoms ($p<0.0001$)
    - Niedzielin ‘01 Eur J Gastro Hep

- Decreased flatulence at end of 4 wk treatment ($p<0.05$)
- Decreased gas production 12 months after trial ($p<0.05$)
    - Nobaek ‘00 AJG

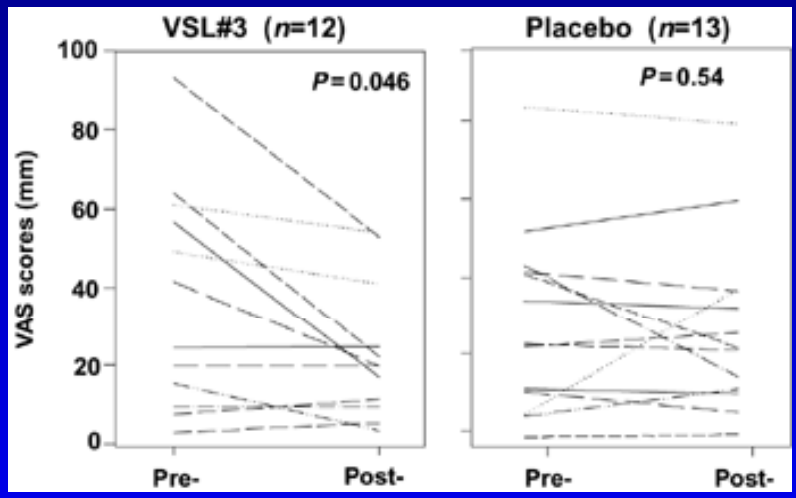
VSL#3

- Composed of 8 species:
  - *Bifidobacterium longum, infantis, and breve*
  - *Lactobacillus acidophilus, casei, bulgaris, and plantarum*
  - *Streptococcus salivarius spp thermophilus*

- Differences in PEP of IBS symptoms vs placebo, not signficant
- Secondary endpoint of bloating was significantly different

- Larger study failed to observe bloating difference, but observed significant decrease in flatulence

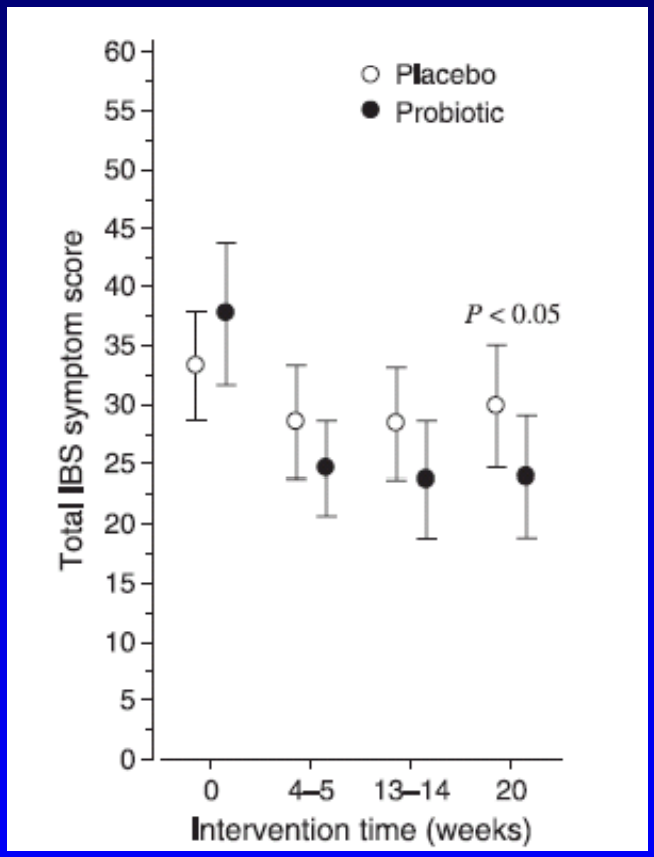
Individual abdominal bloating scores using a visual analogue scale (mm)



Significant improvement in the VSL#3 group ($P = 0.046$), but not in the placebo group ($P = 0.54$).

Kim '03 APT; Kim '05 NGM

Dairy-juice probiotic mix



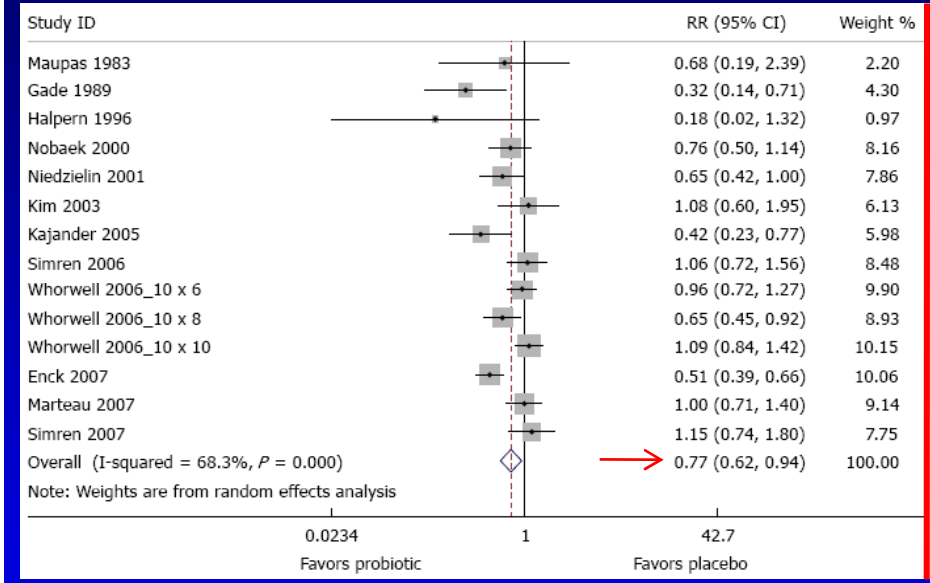
- Composed of 10^7 of each:
  - *L. rhamnosus GG*
  - *L. rhamnosus Lc705*
  - *B. animalis spp lactis Bb12*
  - *Propionibacterium freundenreichii*
- 5 month trial
- Predefined IBS symptom score (abdpain + distention + flatulence + rumbling)
- At wk 20, p=0.0083. No signif differences at earlier times
- Limited by small sample (86)

Kajander '08 APT

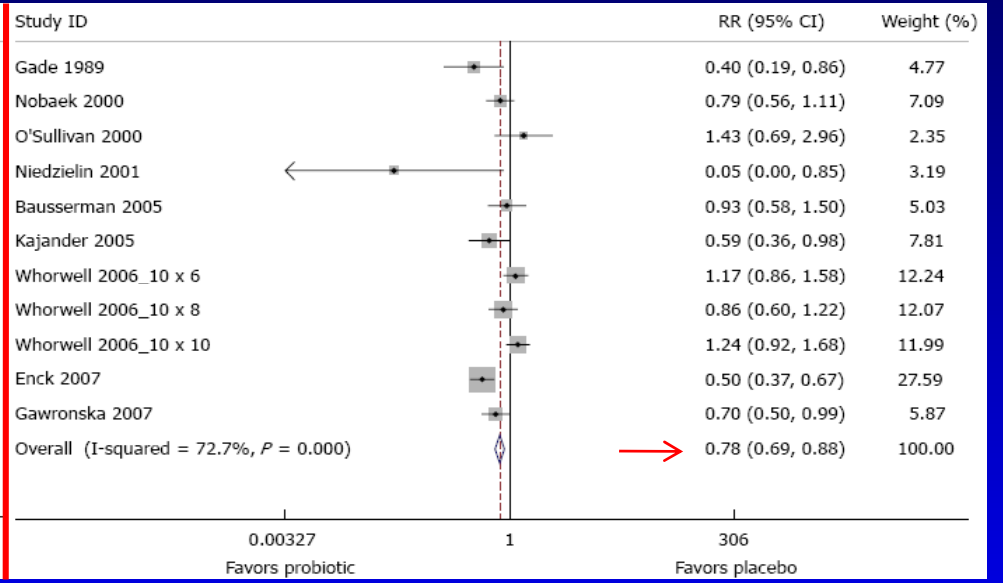
Meta-analysis of probiotics for IBS

MacFarland, WJG '08

12 RCT measuring relative risk of IBS symptoms

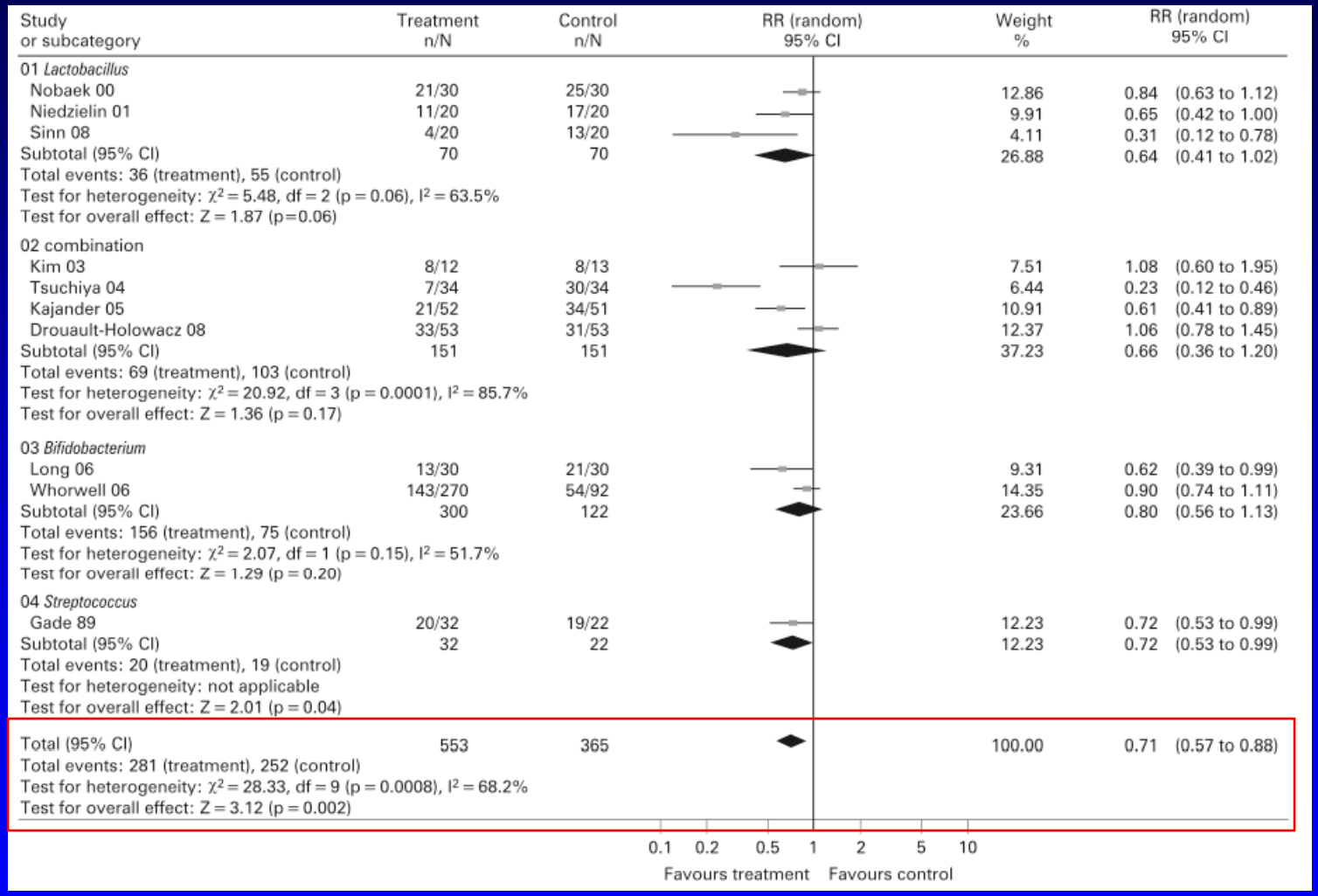


10 RCT measuring relative risk of abdominal pain



- The authors concluded that there are beneficial effects of the use of probiotics in IBS
- Note grouping together all probiotic bacteria under one umbrella despite known differences in characteristics and intestinal effects of different bacteria.
- Large variability in study design, bacteria, dosage, and outcome measures quantitative pooling of data is not feasible

Systematic review: Probiotics for IBS



Variety of species, strains, and doses of probiotics used

Moayyedi, Gut'09

Systematic review: Probiotics for IBS

- Heterogeneity and possible funnel plot asymmetry
  - NNT of 4.
  - Effectiveness likely overestimated
- Higher quality studies reported a more modest treatment effect
  - Trials with Jadad score $\geq$ 4 --> RR = 0.86
  - Trials with Jadad score < 4 --> RR = 0.52
- No significant differences in adverse events between probiotic group and placebo (when reported)

Are probiotics safe?

- Probiotics viewed by many as “natural” alternatives to traditional medicines
- Most papers report AE rates equivalent to controls/placebos

- Recent reports suggest probiotics may present substantial mortality risk to seriously ill or immunocompromised
  - Severe acute pancreatitis ---> increased rate of intestinal ischemia and mortlity in probiotic group ($p < 0.01$) Baseline’08 Lancet
- Non-traditional safety concerns:
  - Unpredictable behavior of naturally occurring microbes
  - Unpredictable behavior of gentically altered microbes
  - Unpredictable interactons of bacteria with human host
  - Unexpected release of novel bacteria into the environment

Take home messages

- Strong scientific and epidemiological studies demonstrate alterations in the gut microbiota can lead to functional GI symptoms --> rationale for probiotics is strong
- Enthusiasm and claims for probiotics exceed the scientific evidence to support its use
- Results are highly species or strain specific, and should not be extrapolated to other bugs, I.e. not all probiotics are the same
- "Best evidence" to date for
  - *Bifidobacterium infantis* 35624 (sold as Align)
  - *Lactobacillus plantarum 299v*
  - VSL#3 (for bloating, flatulence)
- Need large, well-designed controlled trials
- Overall safety record is good; caution in the seriously ill or immunocompromsed patient
- Bar is "set low" to use as adjunctive therapy or for non-responders to traditional therapy