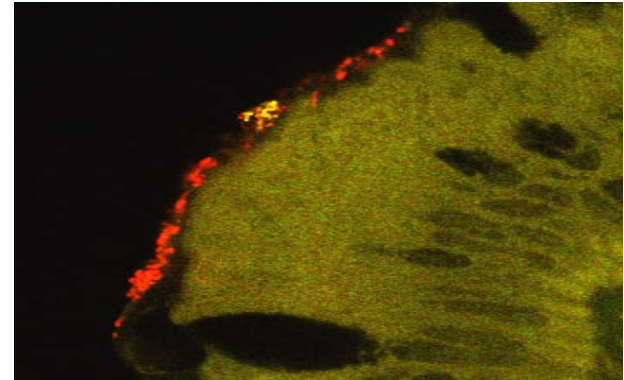


The role of mucus associated bacteria in Inflammatory Bowel Disease

Broad Medical Research Program
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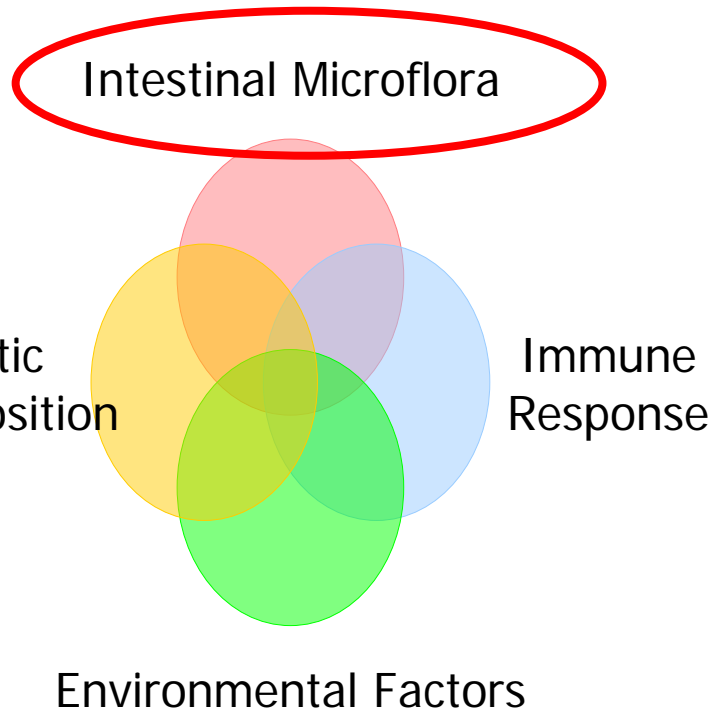
The University of New South Wales

Sydney, Australia

UNSW



The role of microorganisms in IBD



HOWEVER

the microorganisms involved in IBD
remain in dispute

Evidence for a range of microorganisms

Paramyxoviridae

Mycobacterium paratuberculosis

Bacteroides vulgatus

Yersinia species

Fusobacterium varium

Escherichia coli subtypes

NO CONSISTENT DATA

ARE WE LOOKING AT THE WRONG BACTERIA?

If bacteria are involved in the initiation of IBD, the most likely candidates are those closest to the mucosal surface!

- Early studies in rodents

Intestinal mucus & crypts colonised by

Spiral/helical bacteria

Borrelia spp

Treponema spp

Spirillum spp

“Others”

“Others” now known to include:

- Members of *Helicobacteraceae*

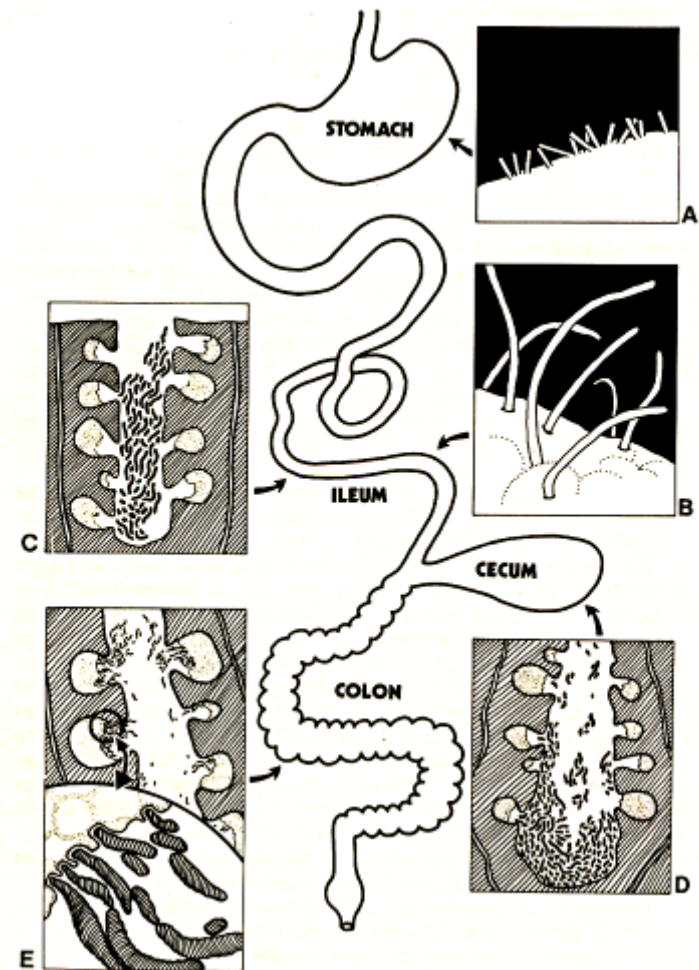
Helicobacter and *Wolinella* genera

Enterohepatic Helicobacters

Gastric Helicobacters

eg. *H. pylori*

- *Campylobacter* species



Aim and study population

- Aim

To investigate the role of members of the *Helicobacteraceae* and *Campylobacter* species with CD

- Study population

Children

Newly diagnosed

Free from many confounding factors

Detection of members of the *Helicobacteraceae* and *Campylobacter* species in biopsy samples

COHORT 1

- Children

- 54 CD (Newly diagnosed)

- 2-16yrs; mean 9.7years

- 57 symptomatic Non-IBD controls (No pathology)

- 2-15yrs; mean 9.4years

- 22 symptomatic Non-IBD controls (Pathology)

- 2-15 yrs; mean 8.6years

- Diagnoses

- Allergic diseases, Colonic eosinophilia

- Coeliac disease, Yersinia infection

- Reflux oesophagitis, Rectal prolapse,

- Active proctitis, Peutz Jeghers syndrome

Materials and Methods

Detection and identification of members of the *Helicobacteraceae* and *Campylobacter* genus in biopsies and fecal samples

- DNA Extraction

Qiagen Mini stool kit

- *Helicobacteraceae* specific PCR and Sequencing

Amplify 16S rRNA gene with *Helicobacteraceae* specific primers

C412F/C1288R (~400 bp) (Riley *et al.* 1996)

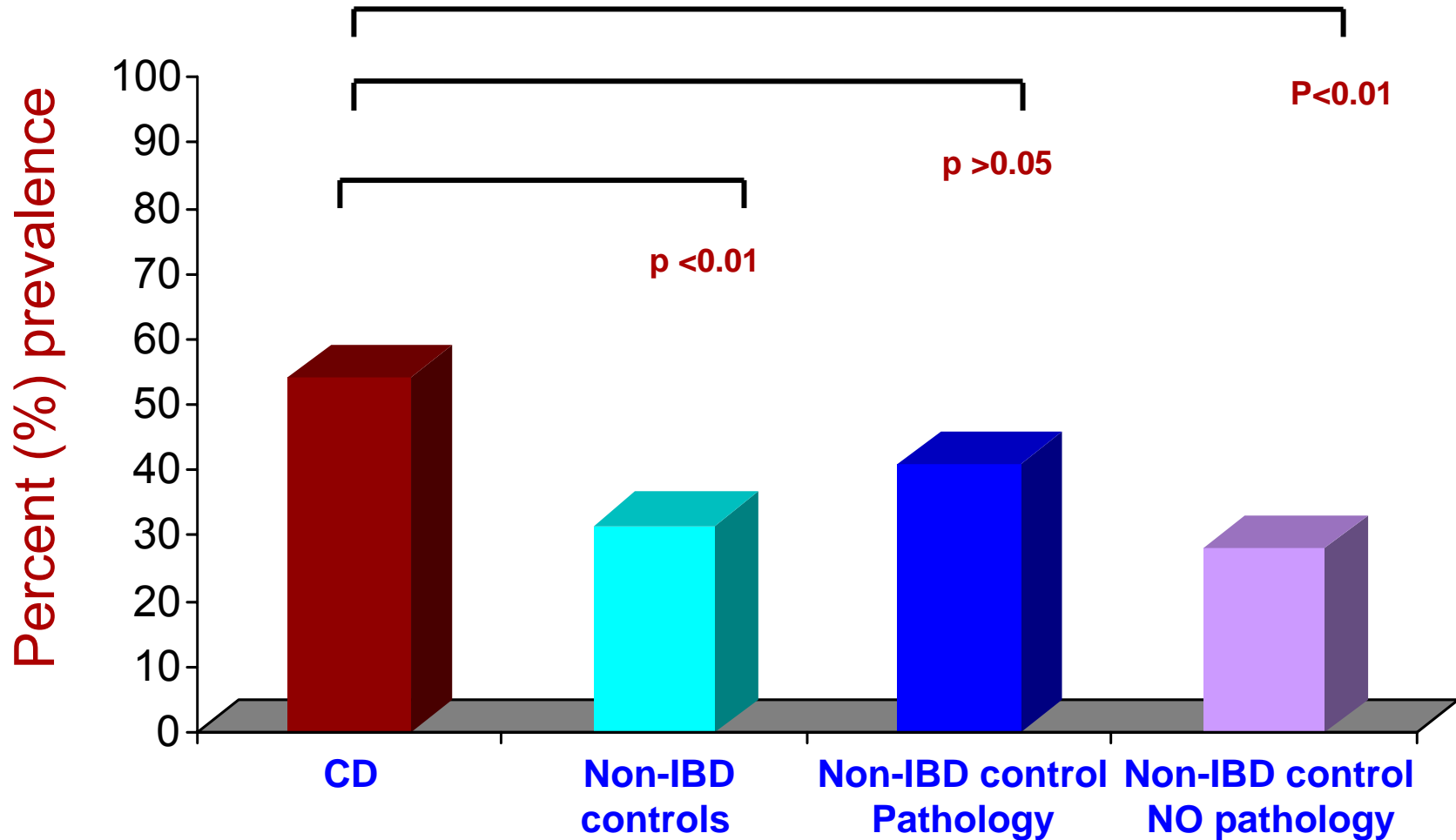
GC658F/1067R (~400 bp) (Grehan *et al.* 2002)

- *Campylobacter* genus-specific PCR and Sequencing

Amplify 16S rRNA gene with *Campylobacter*-specific primers

C412F/C1288R (~800 bp) (Linton *et al.* 1996)

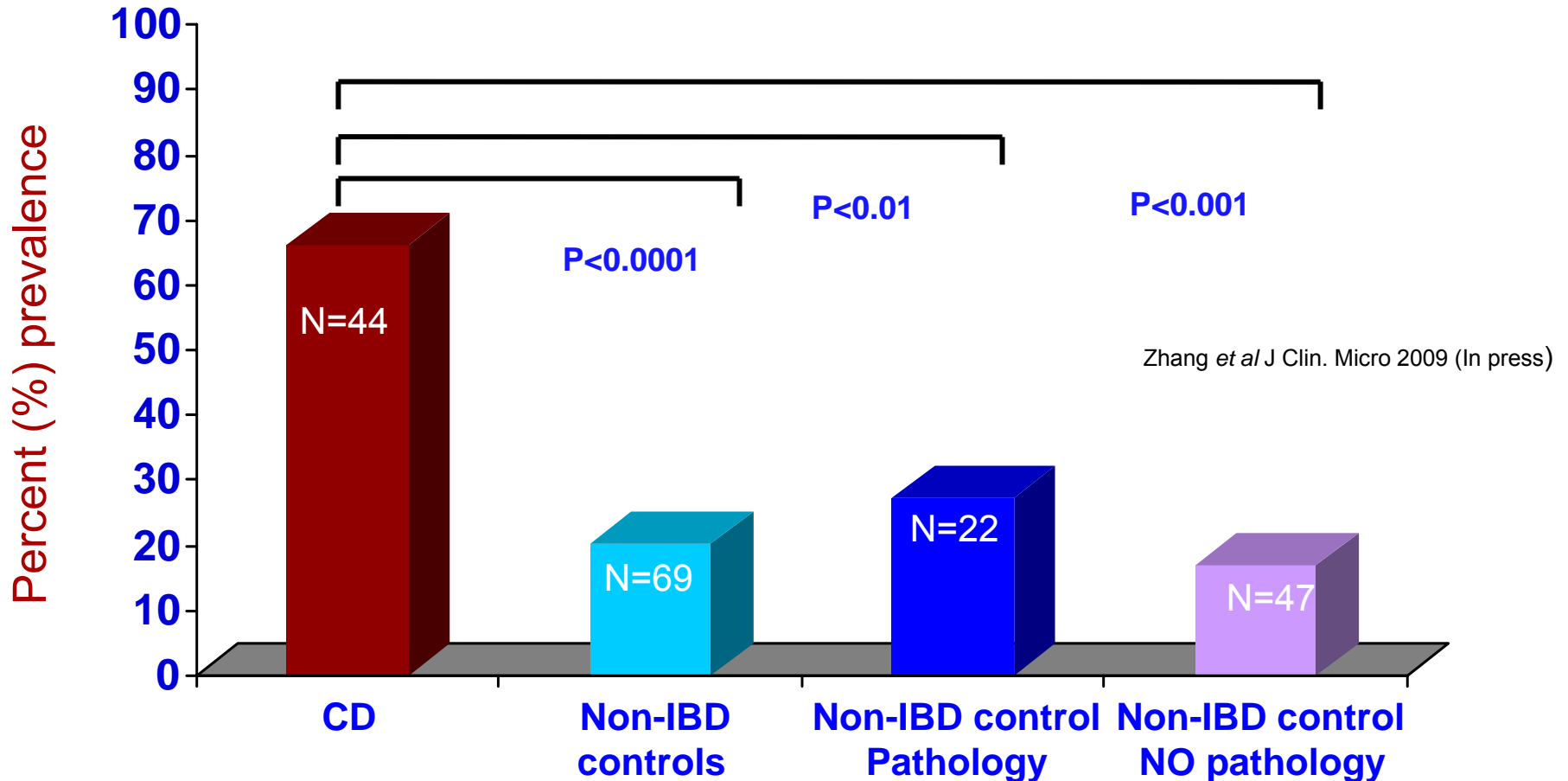
Detection of members of the *Helicobacteraceae* in biopsy specimens using PCR



Summary of sequencing results

Prevalence of non-*pylori Helicobacteraceae* species significantly higher in CD children (32%) when compared with controls (12%) ($p < 0.05$)

A significantly higher prevalence of *Campylobacter* species detected by PCR in biopsy samples from children with CD



Summary of sequencing results

Prevalence of *Campylobacter concisus* significantly higher in CD children (39%) when compared with controls (2%) ($p < 0.0005$)

Detection of members of the *Helicobacteraceae* and *Campylobacter* genus in fecal samples of CD children and controls COHORT 2

- Children

29 CD

Mean age 12.6 years

11 Healthy controls

Mean age 7.2 years

26 Non-IBD controls

Mean age 10.2 years

- Diagnoses

Functional bowel disorders, Reflux esophagitis

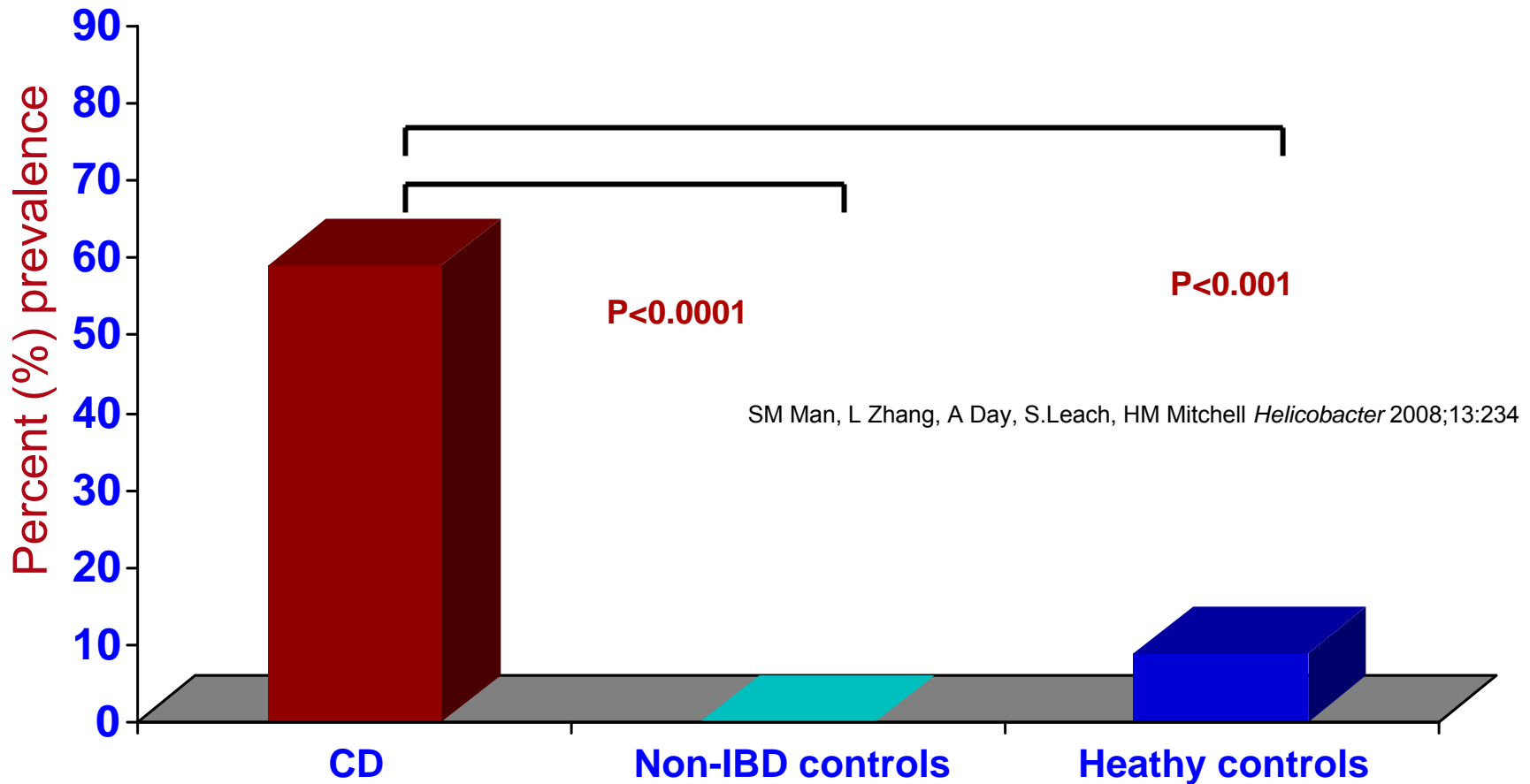
Eosinophilic gastrointestinal disease

Perianal fistula, Anaemia, Systemic inflammatory process,

Duodenal ulcer, *Helicobacter* gastritis, Constipation,

Mild focal cryptitis

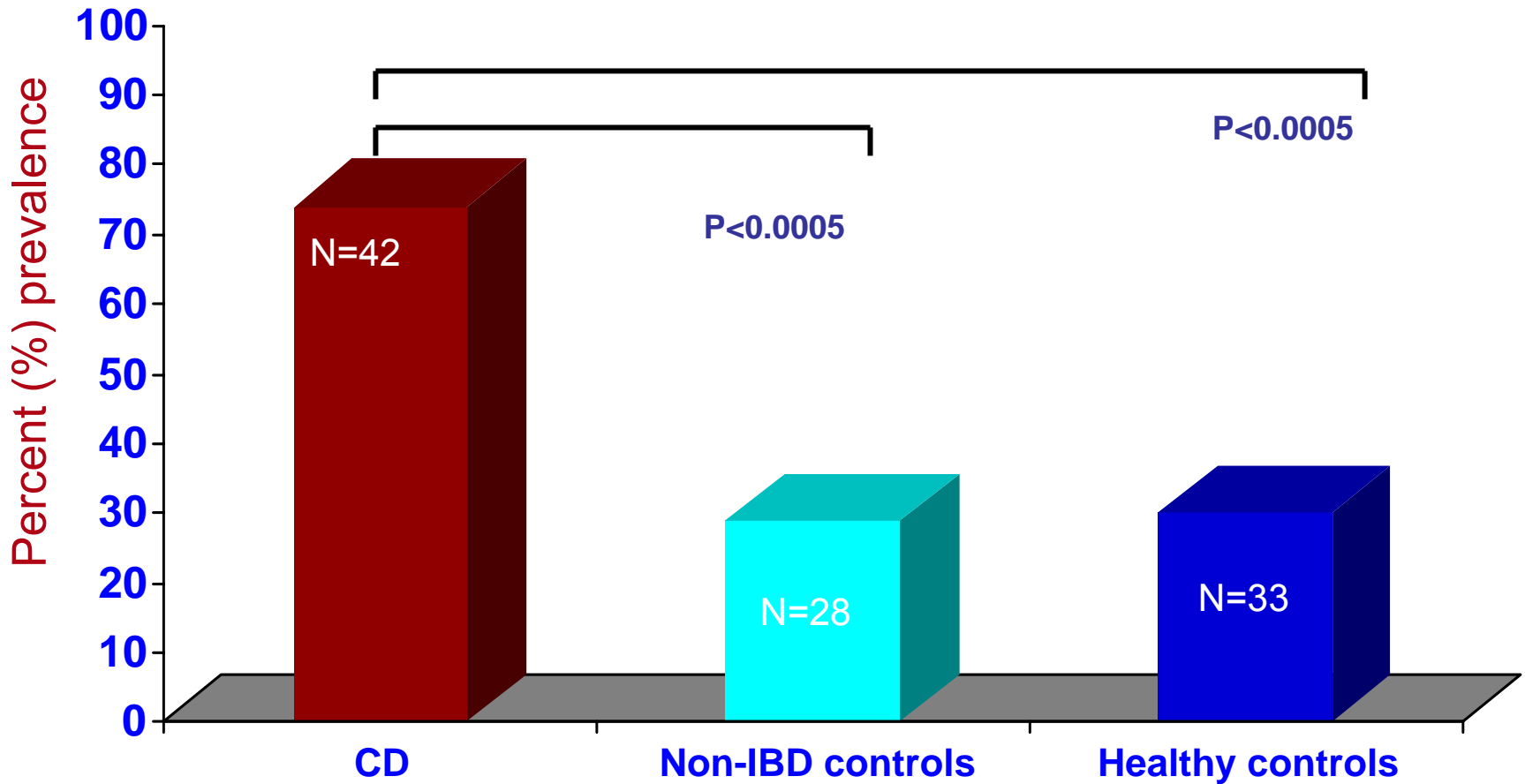
A significantly higher prevalence of *Helicobacteraceae* species detected by PCR in fecal samples from children with CD



Summary of sequencing results

Prevalence of *non-pylori Helicobacteraceae* species significantly higher in CD children (38%) when compared with controls (9%) ($p < 0.001$)

A significantly higher prevalence of *Campylobacter* species detected by PCR in fecal samples from children with CD



Summary of sequencing results

Prevalence of *Campylobacter concisus* higher (31%), but not significantly, in CD children when compared with controls (13%) ($p < 0.05$)

Culture of mucus associated bacteria

- Culture of mucus associated bacteria problematic

Pre-induced diarrhea prior to colonoscopy flushes out intestinal contents

Reduces bacterial load in biopsy?

Fastidious nature of *Helicobacteraceae* and *Campylobacter* genus makes culture difficult - slow growers

- Current success with culturing

Crohn's Disease

H. pylori cultured from 1 child but NOT in pure culture (identified by sequencing)

C. concisus cultured & mixed culture of *H. pylori* (identified by sequencing) from 1 child

C. hominis cultured from 1 child

C. showae cultured from 1 child

B. ureolyticus cultured from 1 child

Non-IBD controls Pathology

C. jejuni cultured from 1 child

B. ureolyticus cultured from 3 children

Non-IBD controls NO Pathology

H. bilis / *H. canis* in mixed culture (identified by sequencing)

B. ureolyticus and *C. jejuni* cultured from 1 child

B. ureolyticus cultured from 1 child

C. hominis cultured from 1 child

Can non-jejuni *Campylobacter* species invade intestinal cells?

● *Campylobacter* invasion assay

Human intestinal epithelial cell line (CaCO₂)

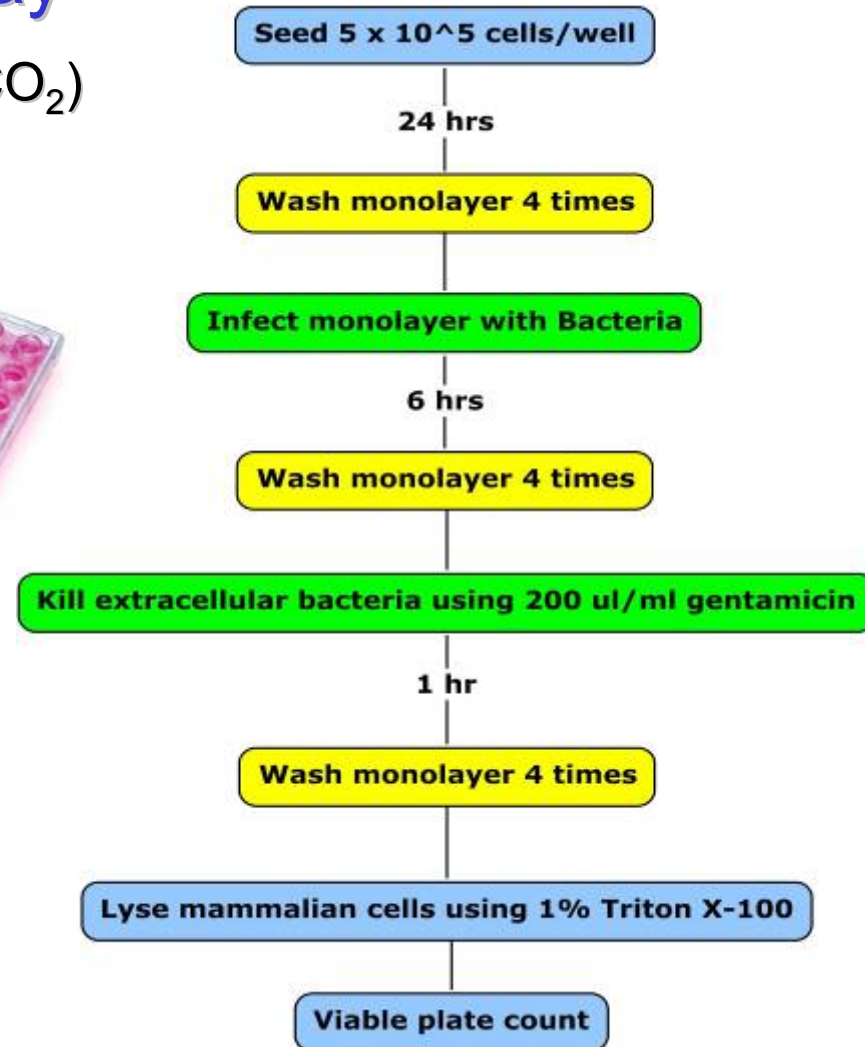
Gentamicin Protection assay

Multiplicity of infection (MOI) = 20

Bacterium
<i>S. typhimurium</i> LT2
<i>C. concisus</i>
<i>C. showae</i>
<i>C. hominis</i>
<i>B. ureolyticus</i>



Bacterium	% invasion
<i>S. typhimurium</i> LT2	0.21
<i>C. concisus</i>	0.01
<i>C. showae</i>	0
<i>C. hominis</i>	0
<i>B. ureolyticus</i>	0

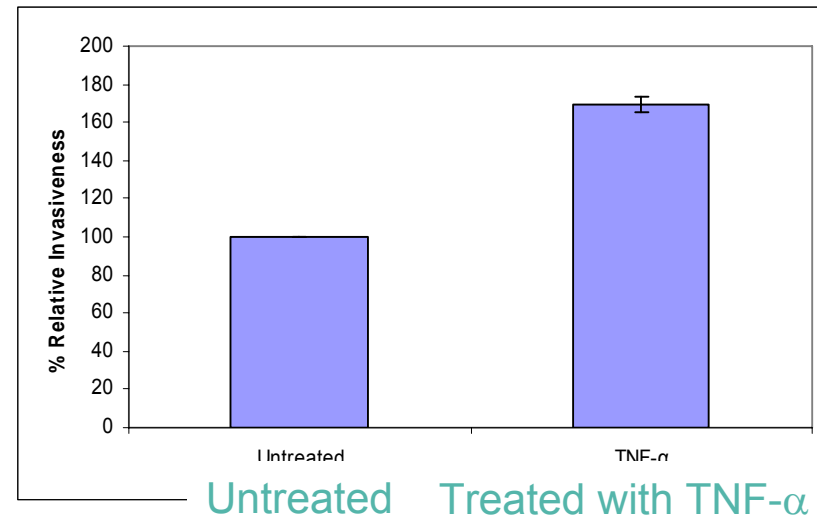


Can *Campylobacter concisus* invade intestinal cells?

Does increasing the MOI improve the ability of *C. concisus* to invade Caco 2 cells?

Does the presence of inflammation increase the invasive ability of *C. concisus*?

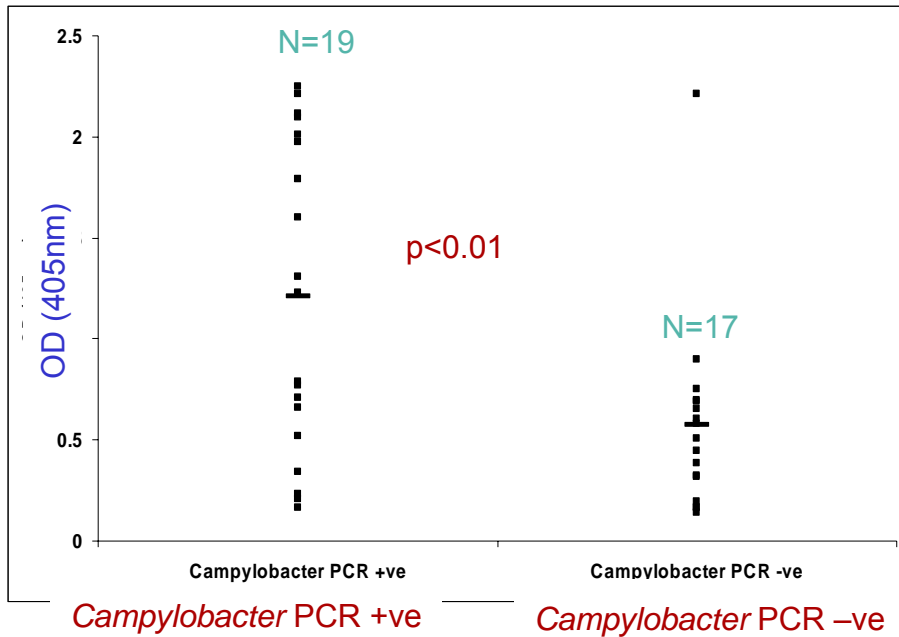
MOI	CFU Invaded (SEM)	CFU Inoculated (SEM)	% Invasion (SEM)
100 (nx3)	$4.6 \times 10^4 \pm 9 \times 10^3$	$3.9 \times 10^7 \pm 5.0 \times 10^6$	0.11 \pm 0.02
200 (nx3)	$9.0 \times 10^4 \pm 3.4 \times 10^4$	$7.4 \times 10^7 \pm 6.0 \times 10^6$	0.13 \pm 0.05



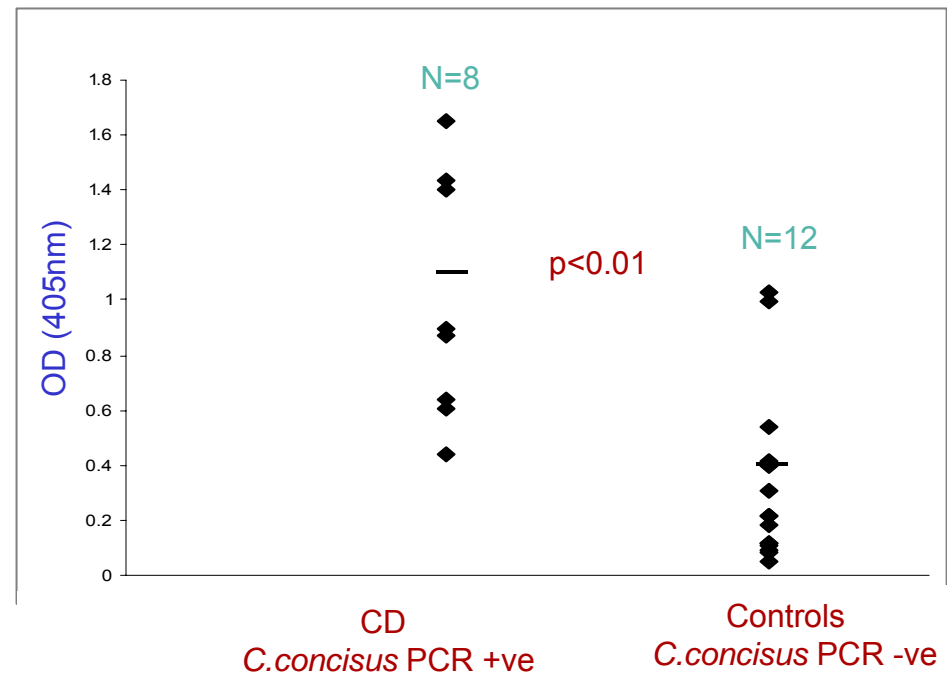
Pre-existing inflammation may increase the ability of *C. concisus* to invade intestinal epithelial cells

Preliminary studies of IgG antibody response to *non-jejuni Campylobacter* species and *Campylobacter concisus*

Non-*jejuni* Campylobacter ELISA



C. concisus ELISA



Significantly higher mean IgG response to *non-jejuni Campylobacter* species and *C. concisus* in children PCR positive for *non-jejuni Campylobacter* species and *C. concisus*

Conclusions

- Increasing evidence that a range of mucus associated bacteria may play a role in the initiation of CD
- Further studies are required to investigate
 - *Pathogenic mechanisms of enteroheptic *Helicobacter* and *Campylobacter* species in CD
 - Mechanism of *C. concisus* attachment to and invasion of intestinal cells
 - *The immune response to mucus associated bacteria
 - *The role of mucus associated bacteria in relapsing CD
 - *Improved culture techniques for enteroheptic *Helicobacter*, *Wolinella* and *Campylobacter* species
 - *Sequencing of pure cultures of *H. pylori* to investigate genetic differences from gastric *H. pylori*

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