Correlates of Protection from *Campylobacter* - Known Unknowns-

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Correlates of Enteric Vaccine-Induced Protection Meeting
Foundation Mérieux
March 22, 2016

The University of Vermont

Over the next 15 minutes

- Very brief background
  - *Campylobacter*, the basics
  - Recent surveillance/observational studies
- Protective immunity. What's implied or observed from:
  - Observation and epidemiologic studies
  - Human Challenge and Homologous Re-challenge Models
- Approaches going forward
- The 'known unknowns'.

Campylobacter: the basics

- In 2010 Global Burden of Disease study, 7.5 million DALYS from *Campylobacter* (#1 bacterial gastroenteritis)
- Second most common cause of bacterial food-borne gastroenteritis in US (1.3m cases of diarrhea/year)
- *C. jejuni* and *C. coli* associated w/ most disease
- Clear associations with long term sequelae: Guillain Barré, reactive arthritis
- Highly strain variable: Especially in surface carbohydrates (LOS core, capsular polysaccharide, locus of flagellin glycosylation)
- No Vaccine: one candidate (capsular PS) in testing

Current Opinion in Gastroenterology 2008, 24:27–31

LOCATION, LOCATION, LOCATION

Developed World

- Sporadic
- More dysentery
- Peak incidence: Young children and young adult
- Usual single pathogen infection
- Adults have an asymptomatic infection every 2 years (asymptomatic: symptomatic ratio, 80-120:1)
- Asymptomatic adults shed/colonized

Developing World

- Often hyper endemic
- More watery diarrhea (except infants)
- Peak incidence: Older infants
- Adults asymptomatic, especially after exposure to multiple strains
- Concurrent infections common
- Colonization/ shedding appears to persist throughout life

GEMS and MAL-ED

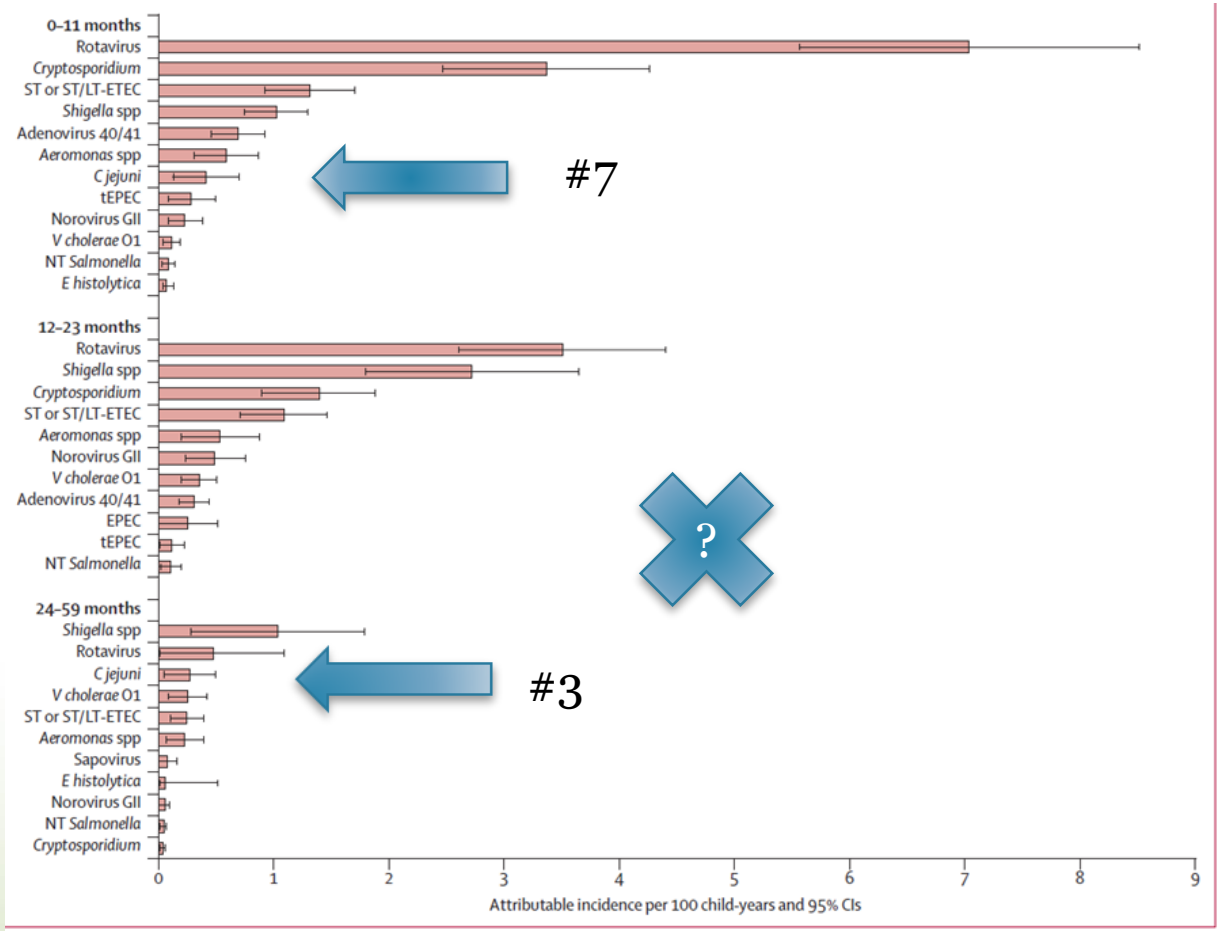

Figure 4: Attributable incidence of pathogen-specific moderate-to-severe diarrhoea per 100 child-years by age stratum, all sites combined
The bars show the incidence rates and the error bars show the 95% CIs.

Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study

www.thelancet.com Vol 382 July 20, 2013

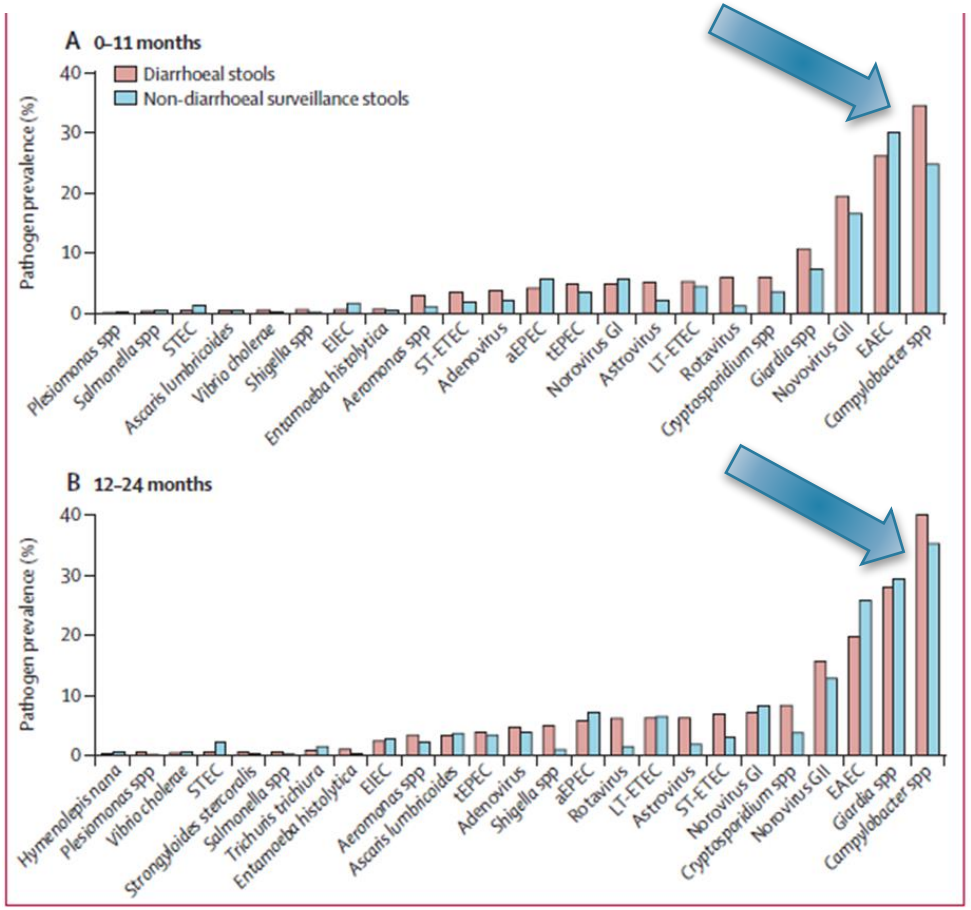

Figure 1: Pathogens detected in diarrhoeal and non-diarrhoeal stools, 0–11 months and 12–24 months

Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED)

www.thelancet.com/lancetgh Vol 3 September 2015

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MAL-ED: age distribution

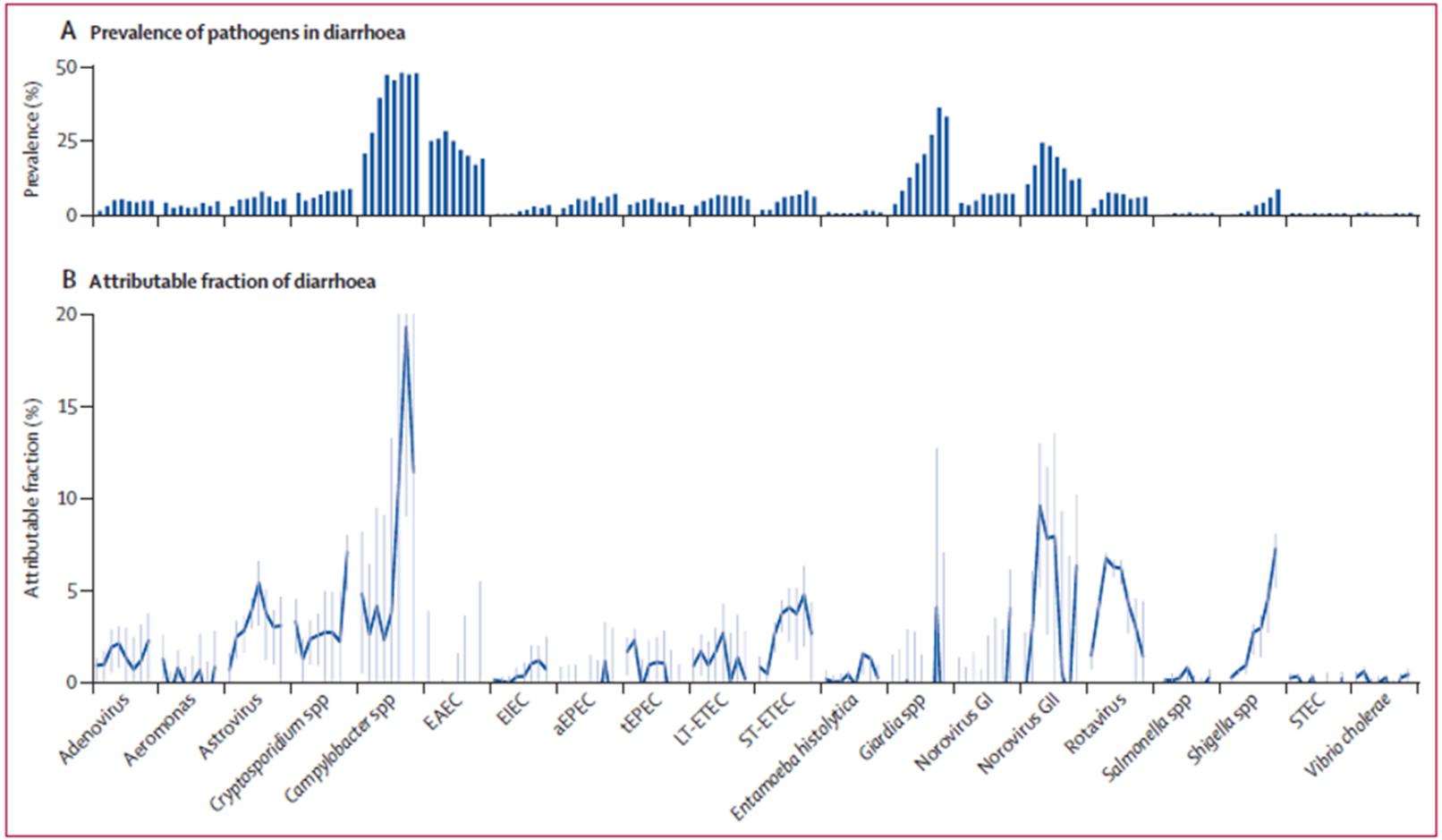

Figure 3: **Prevalence and adjusted attributable fraction of diarrhoea for 3-month intervals, age 0–24 months**
EAEC=enteroaggregative *Escherichia coli;* EIEC=enteroinvasive *E coli;* aEPEC=atypical enteropathogenic *E coli;* tEPEC=typical enteropathogenic *E coli;* LT-ETEC=LT-producing enterotoxigenic *E coli;* ST-ETEC=ST-producing enterotoxigenic *E coli;* STEC=Shiga-toxin producing *E coli*. Data are attributable fractions (95% CI). For each organism, the first data point represents age 0–2 months, the second represents age 3-5 months, then 6–8 months, 9–11 months, 12–14 months, 15-17 months, 18–
onths. .

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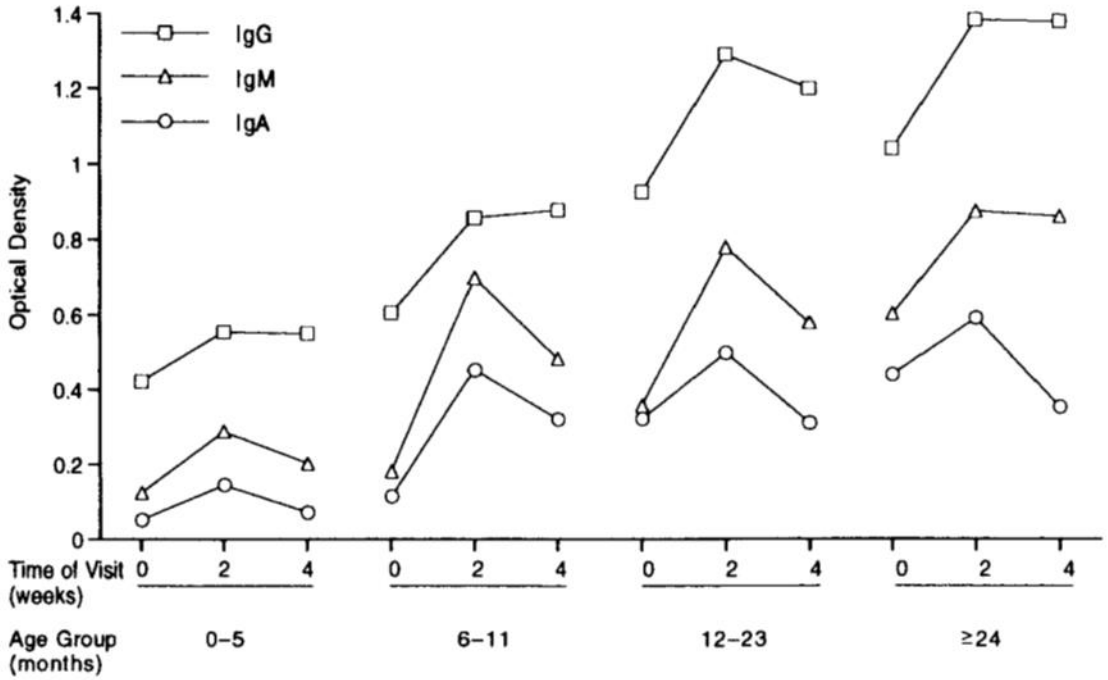
Campylobacter protective immunity: immunocompromised persons

- Not more frequent in persons with IgA deficiency.
- Protracted and severe disease in Hypogammaglobulinemia suggests role of IgG.
- 40X more disease in patients with AIDS. Invasive disease, bacteremia, impaired antibody responses.

Protective immunity: Observational/Epi Studies



In conclusion, in a Thai population in which enteric infections are frequent, *Campylobacter* infections are hyperendemic. As children age, their *Campylobacter* infections become milder, they excrete fewer organisms, and *Campylobacter*-specific serum antibodies rise progressively. All of these findings are consistent with the development of immunity, which suggests that development of a vaccine against *Campylobacter* infections should be possible.

Campylobacter Immunity and Quantitative Excretion Rates in Thai Children

JID 1993;168 (September)

DAVID N. TAYLOR,[1†*] PETER ECHEVERRIA,[1] CHITTIMA PITARANGSI,[1] JITVIMOL SERIWATANA,[1] LADAPORN BODHIDATTA,[1] AND MARTIN J. BLASER[2]

Challenge (re-challenge) Studies

- Goal: vaccine and drug down-selection platform, understanding of correlates of protection (upon re-challenge).
- Caveat: Adults, developed world
- Model Development:
  - A32-49 (1980's): University of MD, CVD
  - 81-176 (1980's to 1999): CVD and US NMRC
  - CG 8421 (2006-present): US NMRC and UVM VTC

Early work with A 3249 and 81-176 (CVD)

- Nine dose-finding studies
  - Difficult to find dose-response relationship.
  - No immunologic exclusions
- Homologous protection shown from diarrhea, not infection at 28d
  - (For 3249, CFU 10^8, n=2; 81-176, CFU 10^9 n=7)
- At re-challenge, no further boost in antibodies
- Observation: those protected from primary disease had somewhat higher baseline IgA

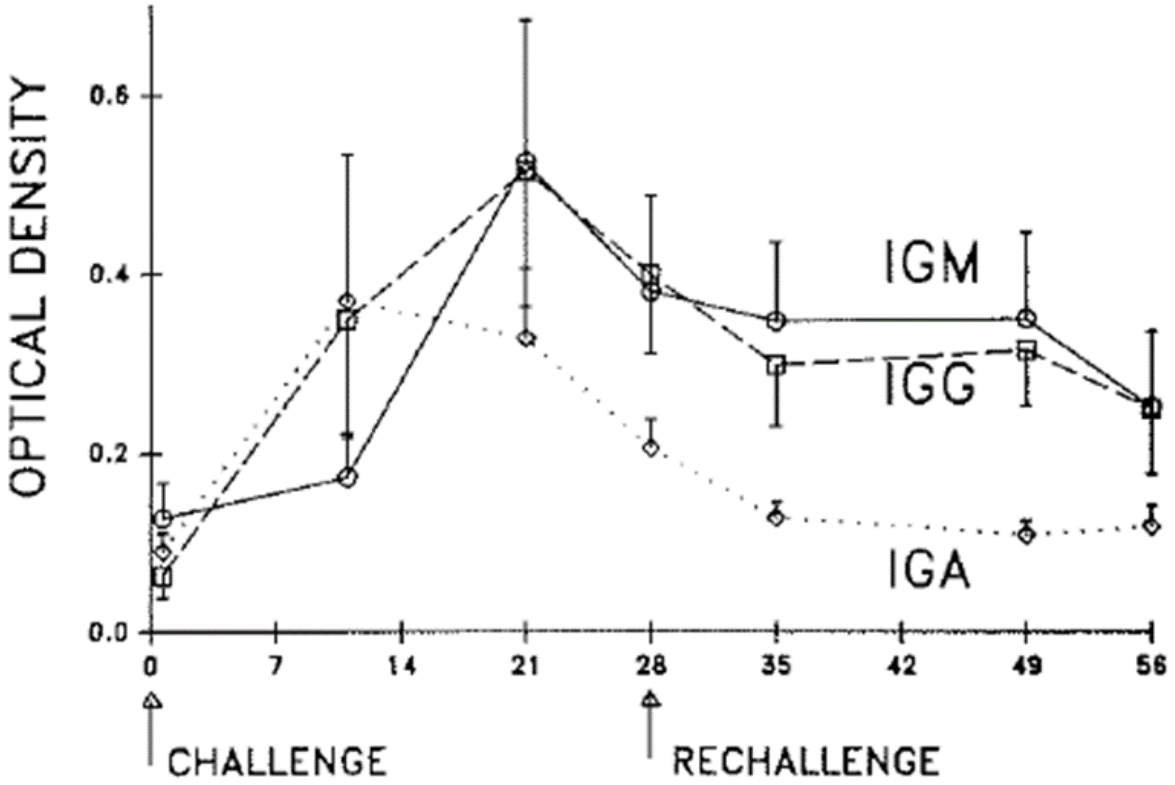


FIGURE 6. Mean serum antibody response to *C. jejuni* acid-extracted proteins following challenge.

Campylobacter jejuni
Current Status and Future Trends

Human Volunteer Studies with *Campylobacter jejuni*

ROBERT E. BLACK, DANIEL PERLMAN, MARY LOU CLEMENTS, MYRON M. LEVINE, AND MARTIN J. BLASER 1992

C. jejuni 81-1

- Subjects with pre-existing IgA excluded (presumably more naïve)
- Improved dose-response at high doses (10^9 CFU): IgA vs. very high dose?
- Re-challenge: 100% homologous protection at 4-6 weeks but 43% at 12 months)
- In primary infection, higher INFγ (in PBMC supernatant) associated with no illness

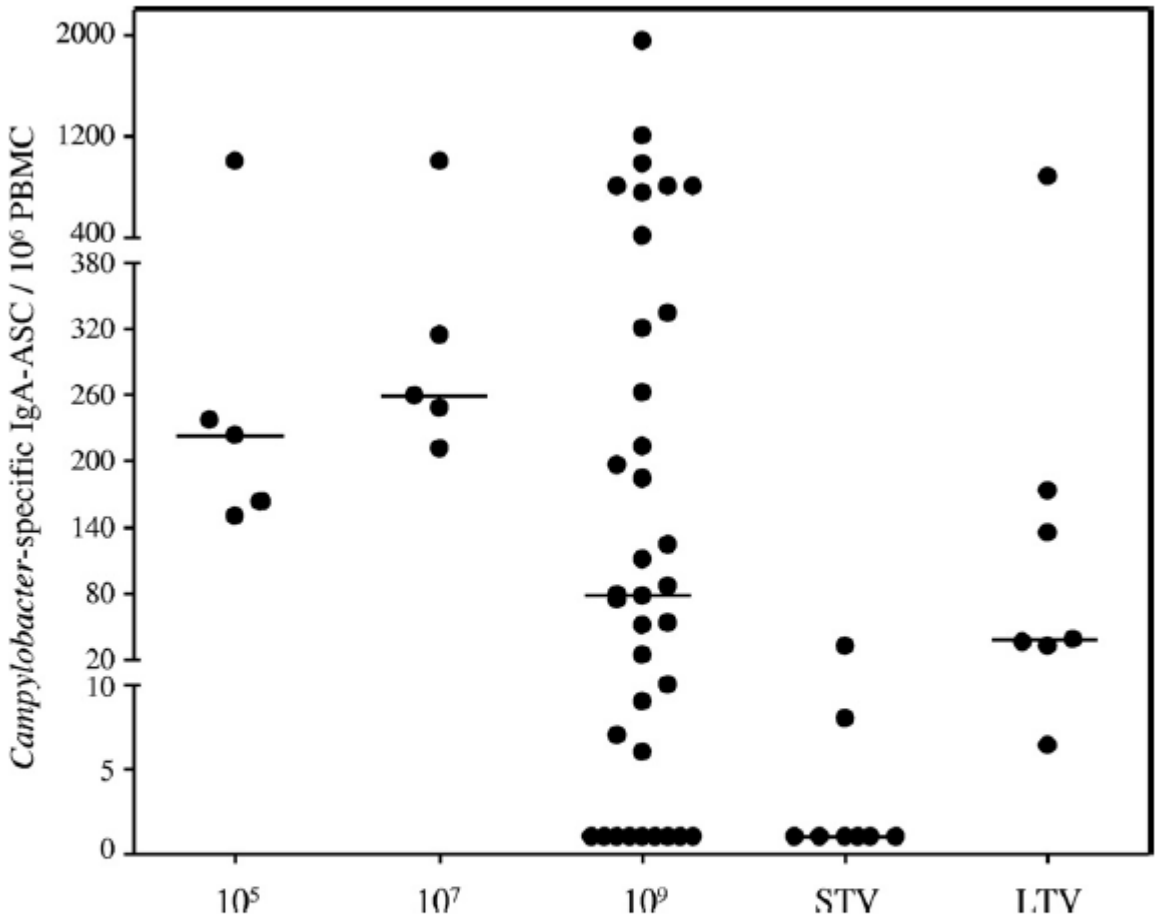


FIG. 3. IgA-specific antibody-secreting cell (ASC) responses following *C. jejuni* infection. The groups studied were naïve subjects who received inocula containing 10^5, 10^7, and 10^9 CFU and STV and LTV who received 10^9 CFU. The response shown are the maximal responses between 6 and 9 days postinoculation. The horizontal lines indicate medians. No ASCs were detected before challenge in any group (data not shown).

INFECTION AND IMMUNITY, Apr. 2010, p. 1750–1759

David R. Tribble,[1]†* Shahida Baqar,[1]† Daniel A. Scott,[1]‡ Michael L. Oplinger,[2]§ Fernando Trespalacios,[2]¶ David Rollins,[1]‖ Richard I. Walker,[4]†† John D. Clements,[3] Steven Walz,[1] Paul Gibbs,[2] Edward F. Burg III,[1]‡‡ Anthony P. Moran,[5] Lisa Applebee,[1] and A. Louis Bourgeois[1]††

"New" CG8421 model (no association GB)

- Excluded if *Campylobacter* specific IgA and/or IFNγ present.
- High, consistent attack rate at 10^{5-6} CFU (86-10%): exclusions vs. new strain?
- Homologous re-challenge performed at 3 month …but 0% protection.

Characteristics of infection (all10^5 CFU)	2007 (n=15)	2010	2010
		1st dose (n=12)	2nd dose (n=8)
Campylobacteriosis, n (%)	14 (93.3%)	14 (93.3%)	8 (100%)
Any diarrhea, n (%)	14 (93.3%)	14 (93.3%)	8 (100%)
Severe diarrhea, n (%)	11 (73%)	8 (53.3%)	7 (87.5%)

C. jejuni CG8421 Human Challenge Model • **CID 2009:49**

C. jejuni Reinfection Fails to Protect • **CID 2013:57 (15 October)**

Immunologic findings w/o protection (CG 8421, 3-month re-challenge)

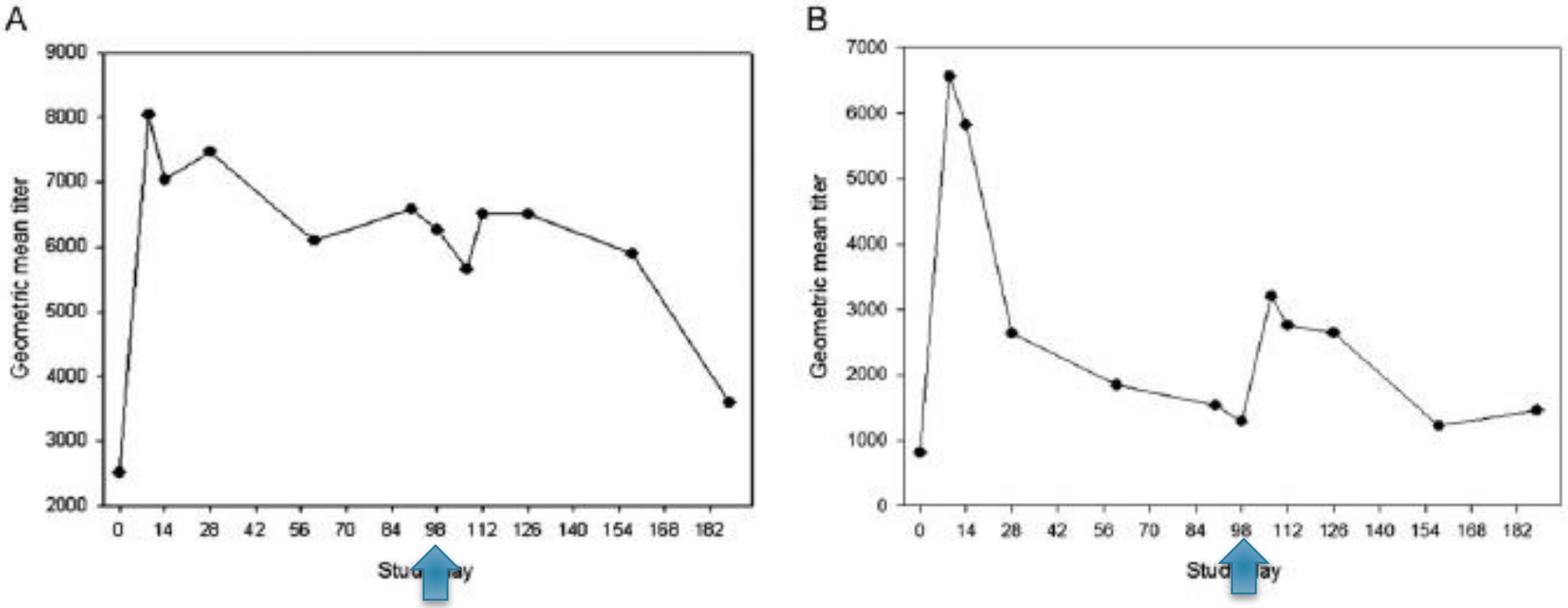


Figure 2 Serum immunoglobulin G (IgG), immunoglobulin A (IgA), and fecal IgA responses after challenge (day 0) and secondary challenge (day 98) with *Campylobacter jejuni* CG8421. *A*, Serum IgG responses after primary (day 0) and secondary (day 98) challenge with *C. jejuni* CG8421. *B*, Serum IgA responses after primary (day 0) and secondary (day 98) challenge with *C. jejuni* CG8421. *C*, Fecal IgA responses after primary (day 0) and secondary (day 98) challenge with *C. jejuni* CG8421. Hatched lines represent total fecal IgA. Solid lines represent *C. jejuni* antigem-specific fecal IgA. Abbreviation: IgA, immunoglobulin A.

Beth D. Kirkpatrick,[1] Caroline E. Lyon,[1] Chad K. Porter,[2] Alex C. Maue,[2] Patricia Guerry,[2] Kristen K. Pierce,[1] Marya P. Carmolli,[1] Mark S. Riddle,[2] Catherine J. Larsson,[1] Douglas Hawk,[2] Elizabeth A. Dill,[1] A. Fingar,[1] Frederic Poly,[2] Kelly A. Fimlaid,[1] Fahmida Hoq,[2] and David R. Tribble[3]

8421 re-challenge.
No ASC boost. No protection

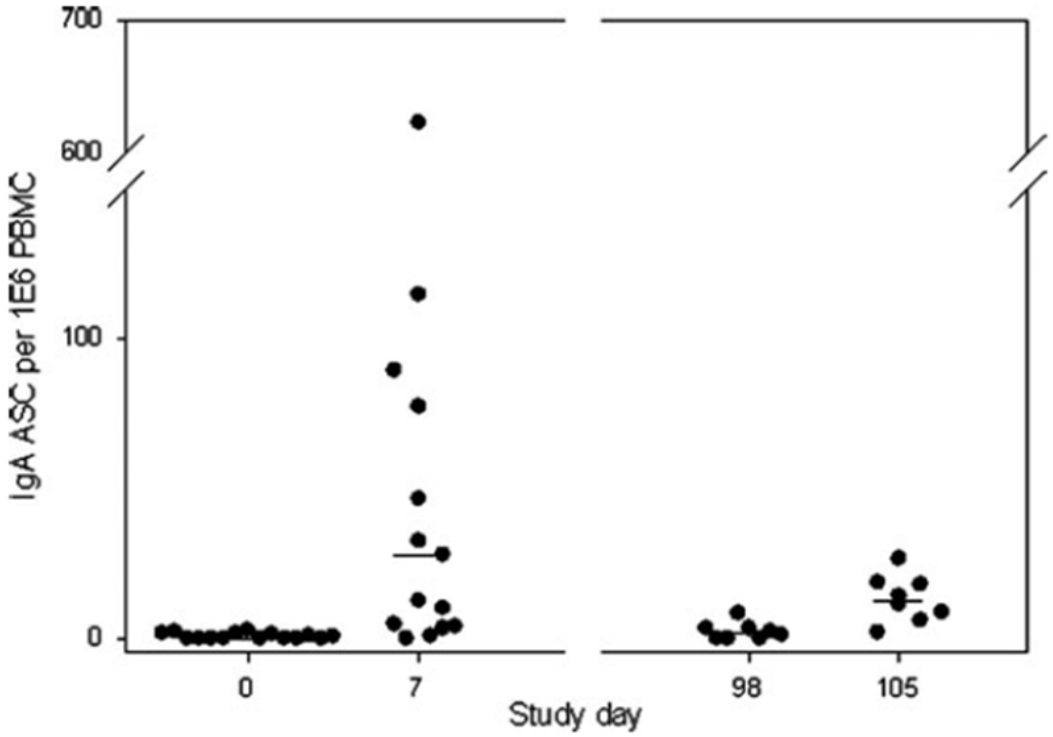
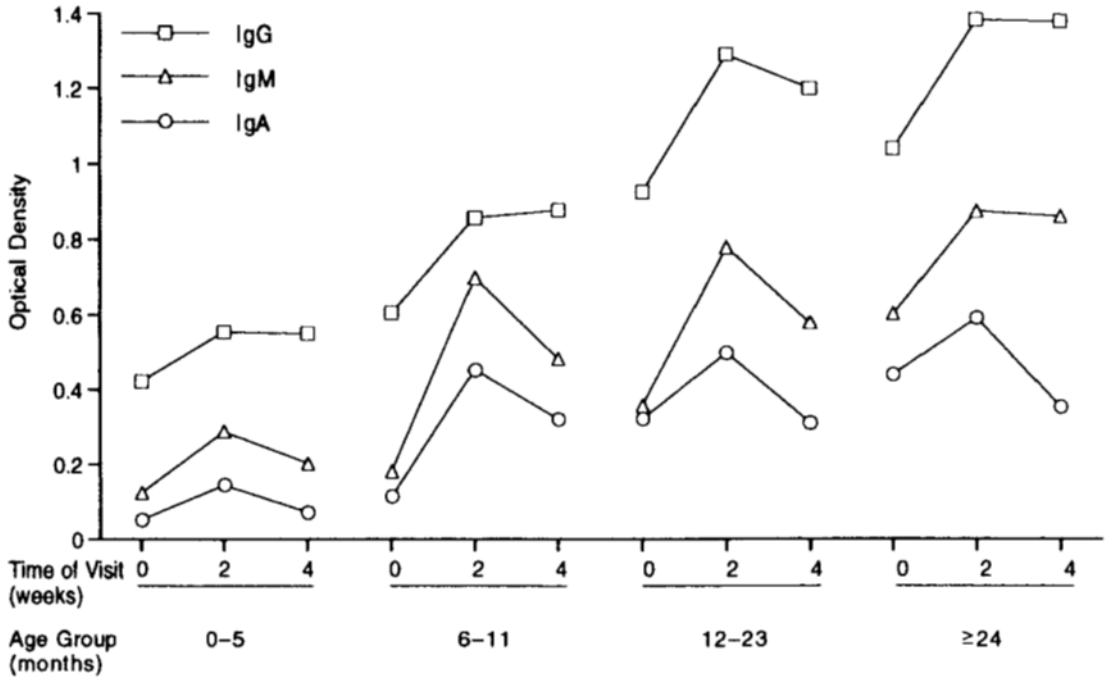


Figure 3. Immunoglobulin A antibody-secreting cell responses after primary and secondary challenge with *Campylobacter jejuni* CG8421. Horizontal lines denote the median number of antibody-secreting cells for each day. Abbreviations: ASC, antibody-secreting cell; IgA, immunoglobulin A; PBMC, peripheral blood mononuclear cell.

Protective immunity: Observational/Epi Studies



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Frequent exposure, especially to heterologous strains is assumed

Campylobacter Immunity and Quantitative Excretion Rates in Thai Children

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Immune protection from *C. jejuni*, recap:

- Natural protection is very short lived to homologous strains.
- Clinical protection from disease in developing world settings is likely seen after several heterologous exposures. Persistence likely requires frequent re-exposure
- Partially understood association of protection with systemic IgA, IgG, IgA ASC (and maybe INFγ)
- No sterilizing immunity develops- asymptomatic infection and shedding persists throughout life
- Currently used immunogenity markers (systemic IgA, IgG, ASC, and INFγ (PBMC supe) are non-specific, a mix of acid-extracted antigens or killed whole-cell bacteria are used as a stimulant. Non specific

Function: how might these IgA and/or IgG antibodies be protective?

- Antibody-mediated opsonization, phagocytic uptake and killing" (*C. jejuni* and *"C. pylori"*)Bernatowska 1989)
- Monocyte/macrophage phagocytic killing (Wassenaar, 1997)
- Strain-specific, complement-mediated modest bactericidal killing (Penne 1986)
- Proposed bacterial agglutination at mucosal surfaces (sIgA)
- Based on surface composition (capsule, sialylation of LOS), complement-mediated killing/antibody function may vary with strain.

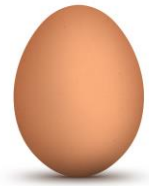
Gut, 1989, **30**, 906–911 E BERNATOWSKA*, P JOSE, H DAVIES, M STEPHENSON, AND D WEBSTER

INFECTION AND IMMUNITY, June 1986, p. 702–706 ROSS A. PENNIE,[1]† RICHARD D. PEARSON,[1] LEAH J. BARRETT,[1] HERMY LIOR,[2] AND RICHARD L. GUERRANT[1]

In the absence of an appropriate animal model

1. Develop effective vaccine

START with #1 or #2?



2. Define mechanism of immune protection from natural disease

Determine correlate of vaccine-induced efficacy (may not be mechanistic)

Campylobacter: The Known Unknowns

- We do not know a/the specific correlate(s) of protection. Current markers of immunogenicity (IgA, IgG, IFNγ) may not be (mechanistic) COP.
- Best revisions challenge and re-challenge models to confirm COP in humans? Likely difficult in developing world settings (co-infections, constant exposure)
- Isotype-specific antibody function? Development of Memory B cells?
- The importance of heterologous infection needs to be better understood. Need to understand if breadth (antibodies to multiple epitope) vs. depth (affinity maturation of conserved epitopes) is most important.
- How to design a vaccine to extend clinical protection (improve on mother nature).
- Can an empiric vaccine be developed in the absence of better understanding of the development of protective immunity?

Lots to Discuss!
Thank you and acknowledgements

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Chad Porter, PhD
Patricia Guerry, PhD
Frederique Poly, PhD

Nick Mantis, PhD

The University of Vermont

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Supplemental slides to follow

Clinical features *Campylobacter*: Developing world (single pathogen)

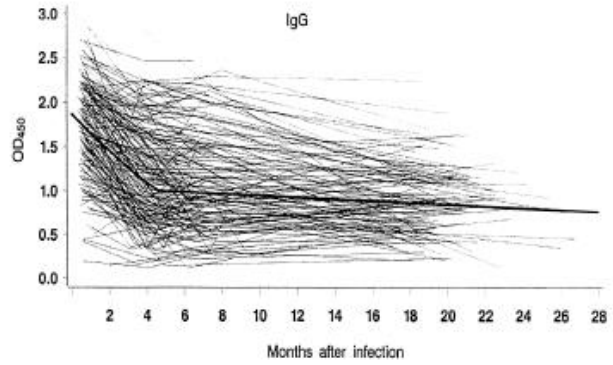
TABLE 3. Clinical features of diarrheal disease in Thai children infected with a single enteric pathogen, Bangkok, Thailand[a]

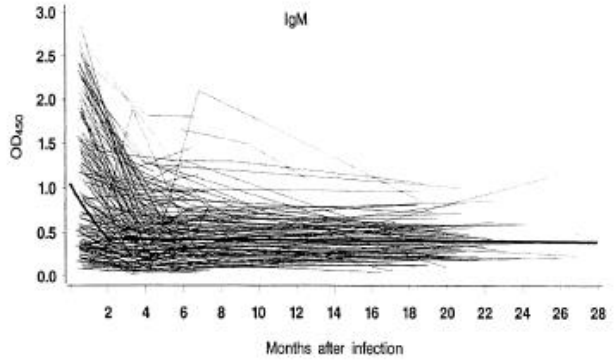
Organism	No. of patients with disease							
	Total no. (%)		No. with single pathogen	No. with fever >38°C	No. with bloody stool	No. with >10 leukocytes	No. given i.v. fluid[b]	No. hospitalized
Shigella spp.	155	(13)	94	42[c]	37	67	3	2
EIEC[d]	19	(2)	11	45	18	36	9	0
Salmonella spp.	151	(12)	81	27	26	27	3	4
Campylobacter spp.	163	(13)	80	28	14	24	4	0
Rotavirus	220	(20)	141	28	5	6	19	4
ETEC[d]	112	(9)	48	17	4	6	6	2

[a] Adapted from reference 12.
[b] i.v., intravenous.
[c] Expressed as percentage of single-pathogen infection.
[d] EIEC, enteroinvasive *E. coli*; ETEC, enterotoxigenic *E. coli*.

Antibody Responses to *Campylobacter* Infections Determined by an Enzyme-Linked Immunosorbent Assay: 2-Year Follow-Up Study of 210 Patients

METTE AAGAARD STRID,[1] JØRGEN ENGBERG,[1] LENA BRANDT LARSEN,[1] KAMILLA BEGTRUP,[2] KÅRE MØLBAK,[1,2] AND KAREN ANGELIKI KROGFELT[1]*





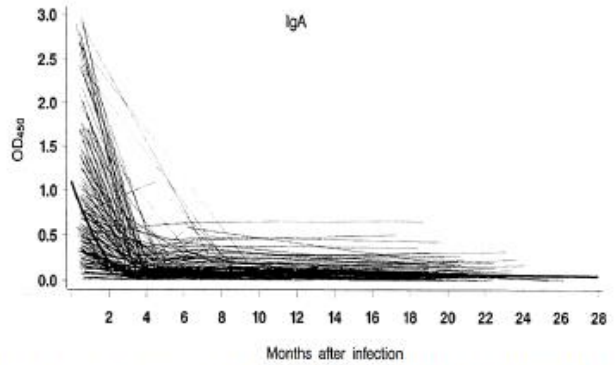


FIG. 1. Serum antibody response to *Campylobacter* infection in patients. (A) IgG; (B) IgM; (C) IgA. Individual responses of 210 patients over a 2-year period according to immunoglobulin class and the fitted population average (bold line) are shown.

CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 2001, p. 314–319