Development, use and refinement of enterotoxigenic *Escherichia coli* (ETEC) controlled human infections

Chad Porter, PhD, MPH
Enteric Diseases Department
Naval Medical Research Center

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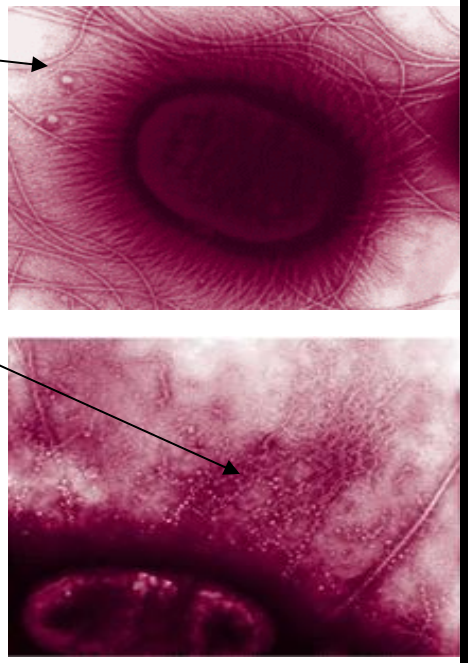
Presentation overview

- Background on ETEC challenge model
- Current state of model
- Refinements to the model
  - Host
  - Strain preparation
  - Disease characterization
  - Non-clinical endpoints
- Next steps in model development and application

Enterotoxigenic *E. coli* (ETEC)

- Epidemiology
  - Common cause of diarrhea in children in low-middle income countries
  - Common cause of travelers' diarrhea
- Clinical symptoms
  - Watery diarrhea
  - Can be accompanied with fever and vomiting

- Colonization factors
  - CFA/I
  - CS1
  - CS2
  - CS3
  - CS4
  - CS5
  - CS6 …



Adapted from: Kaper et al, *Nature* 2004; 2 (2)

- Toxin expression
  - Heat labile (LT)
  - Heat stabile (ST)
  - Both LT and ST

ETEC: The first human challenge

The New England
Journal of Medicine

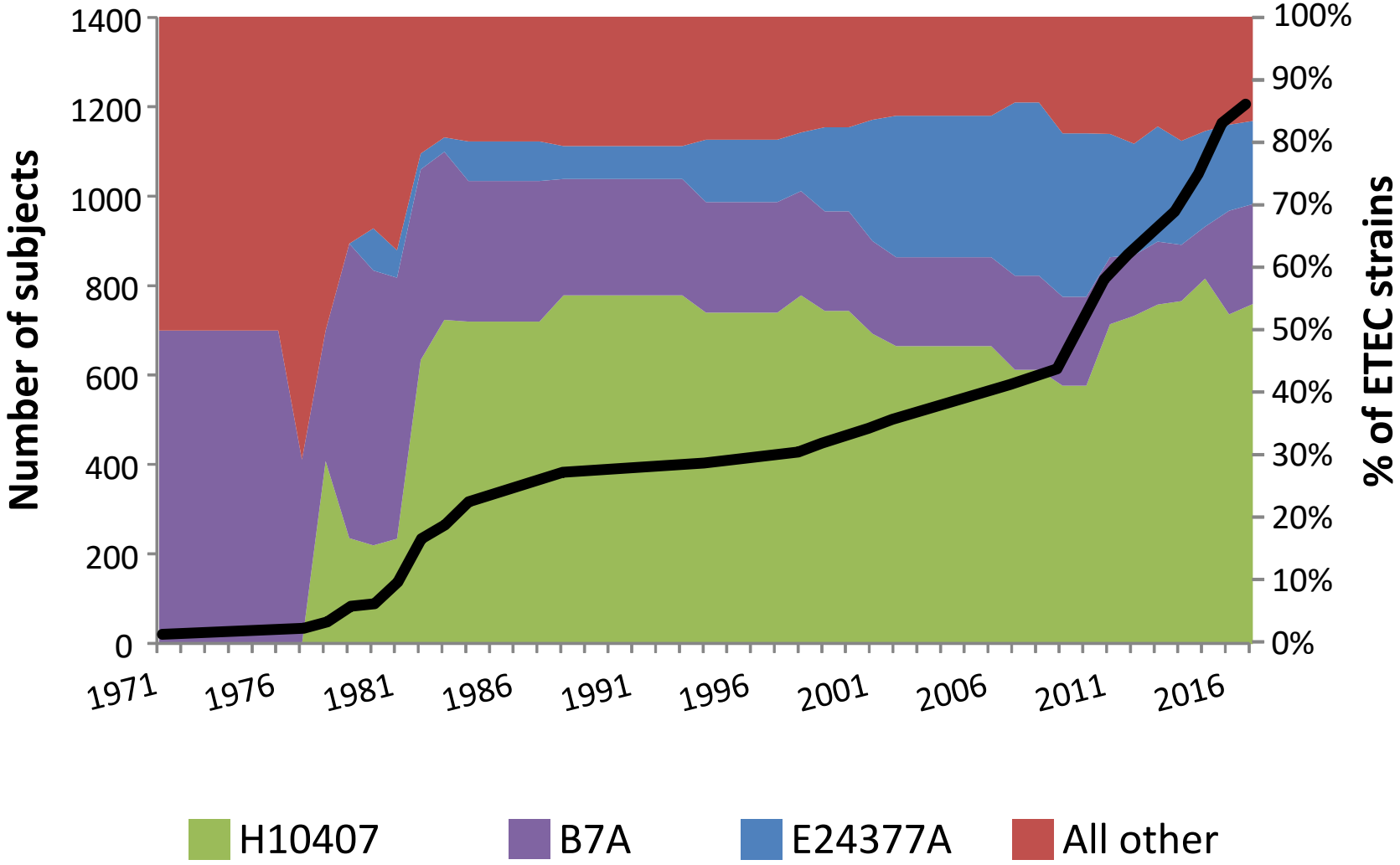
Volume 285 JULY 1, 1971 Number 1

PATHOGENESIS OF *ESCHERICHIA COLI* DIARRHEA

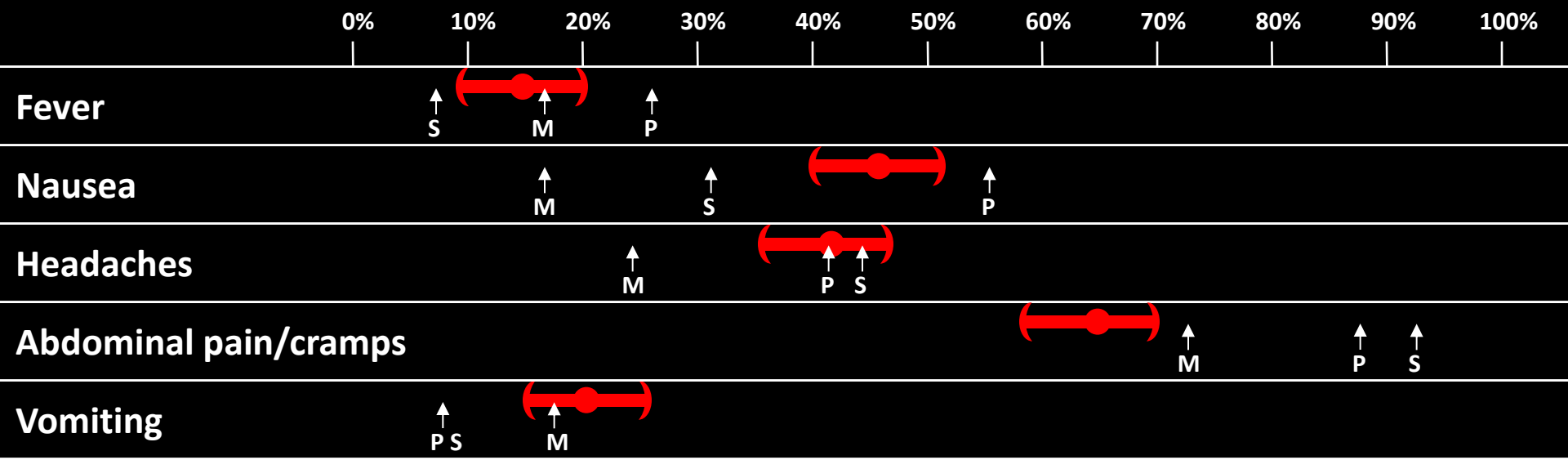
HERBERT L. DUPONT, M.D., SAMUEL B. FORMAL, PH.D., RICHARD B. HORNICK, M.D., MERRILL J. SNYDER, PH.D., JOSEPH P. LIBONATI, PH.D., DANIEL G. SHEAHAN, M.B., M.SC., EUGENE H. LABREC, PH.D., AND JOHN P. KALAS, M.D.

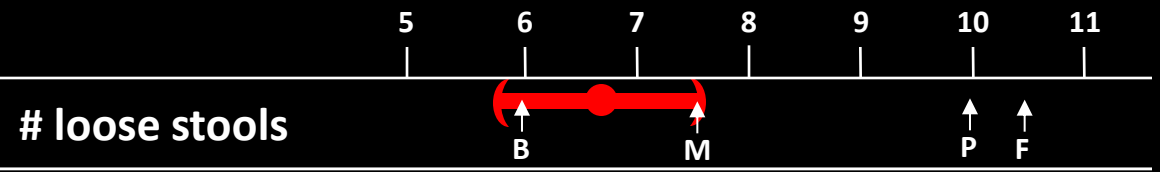
'The results of the studies reported here emphasize that strains of Esch. coli can cause disease in man…' '…organisms that do not possess invasive capabilities but do produce an enterotoxin may be associated with a diarrheal syndrome that resembles clinical cholera.'

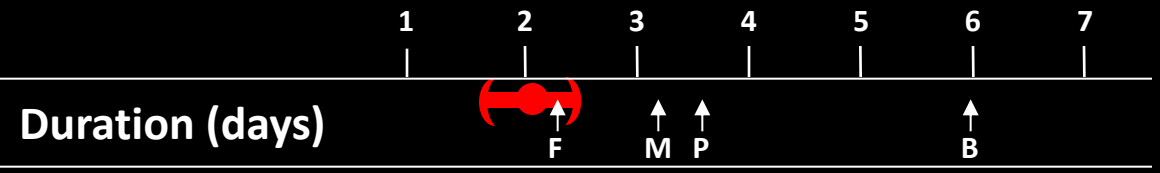
ETEC challenges (1971-2017)



Similarities to natural infection







= Challenge model point estimate and 95% CI

ID	PI	Reference	Region
F	Frech	Lancet 2008	Mexico / Guatemala
P	Porter	DMID 2009	Turkey
B	Bölin	JCM 2006	Mexico / Guatemala
M	Matilla	CID 1994	Morocco
S	Sanders	AJTMH 2002	Thailand

Updated from: Vaccine 29 (35): 5869-5885

‘Standard’ model characteristics

Inclusion / Exclusion

- 18 to 50 year old male or non-pregnant female
- Occupational exposure to ETEC or cholera
- Symptoms of travelers’ diarrhea during travel to ETEC endemic countries
- Vaccination for or ingestion of ETEC, cholera or LT toxin

Antibiotic treatment

- Planned: 5 days post-inoculation
- Early treatment if indicated

Discharge

- Feeling well
- Culture negative (x2)

Strain preparation

- GMP cell bank
- Freshly harvested cells diluted to target OD

Host preparation

- Pre/Post-challenge fast
- 90 minute post-challenge fast

Definitions

- Diarrhea (maximum 24 hr ‘loose stool’ output)
  - Mild: 1-3 of 200-400g
  - Moderate: 4-5 or 401-800g
  - Severe: ≥ 6 or > 800 g

  } **Primary outcome**
- Fever
  - Mild: ≥100.4 °F and ≤101.1 °F
  - Moderate: ≥101.1 °F and ≤102.0 °F
  - Severe: >102 °F
- Vomiting (maximum 24 hr episodes)
  - Mild: 1x
  - Moderate: 2x
  - Severe: ≥2x
- Subjective symptoms (nausea, abdominal cramps, malaise, etc)

Past ETEC Challenge Model Applications

Product type	Product	Challenge Strain	Placebo N	Placebo AR (%)	Treatment N	Treatment AR (%)	Efficacy (%)	Field efficacy (%)
Prophylaxis	Bismuth	H10407	16	56	15	13	77	62-65
"	Bovine milk IgG	E24377A	10	30	10	50	-67[a]	0
Antibiotic Rx	Bactrim	H10407	11	--	10	--	50[b]	50[b]
Live vaccine	E1392/75-2A	E24377A	6	100	12	25	75	--
"	PTL-003	E24377A	13	81	13	76	6	--
"	ACE 527	H10407	27	70	29	52	27	--
"	ACE 527+dmLT	H10407	31	68	13	23	66	--
Whole-cell	Formalin-killed	E24377A	10	60	4	50	17	--
"	Colicin-killed	H10407	8	89	10	20	78	--
		B2C	Historical		8	75	75	--
		Unknown	Historical		8	75	75	--
Subunit vaccine	Type 1 pili (IM)	H10407	7	100	6	33	67	--
		H10407	8	88	6	50	43	--
		H10407	11	27	4	75	-177	--
		B7A	6	67	8	63	6	--
"	LT (Transcutaneous)	E24377A	20	75	27	82	-9	11[c]
"	CfaE	H10407	43	56	41	39	30	--

[a]Specific to this formulation as other formulations repeatedly showed high efficacy (60-100%) in experimental challenge; [b]Reduction in duration and 'illness severity'; [c]Limited to LT+ST+ strains

Host preparation

CLINICAL AND VACCINE IMMUNOLOGY, Oct. 2011, p. 1719–1727 Vol. 18, No. 10
1556-6811/11/$12.00 doi:10.1128/CVI.05194-11

Refinement of a Human Challenge Model for Evaluation of Enterotoxigenic *Escherichia coli* Vaccines[▽]

Clayton Harro,[1]* Subhra Chakraborty,[1] Andrea Feller,[1] Barbara DeNearing,[1] Alicia Cage,[1] Malathi Ram,[1] Anna Lundgren,[2] Ann-Mari Svennerholm,[2] August L. Bourgeois,[3] Richard I. Walker,[3] and David A. Sack[1]

Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland[1]; Department of Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden[2]; and PATH, Washington, DC[3]

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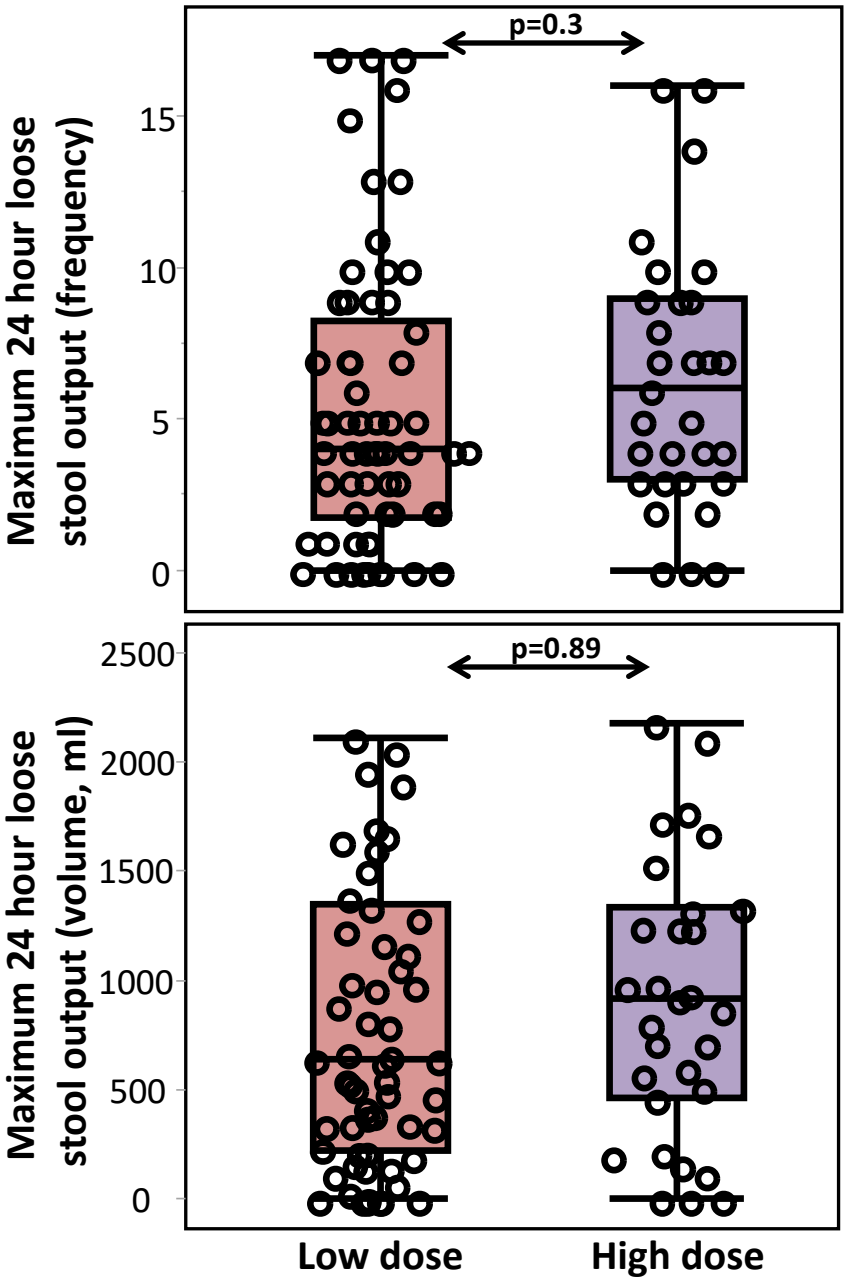
Overnight fast

H10407

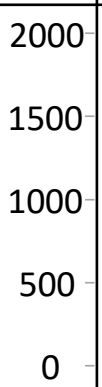
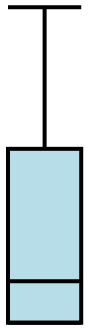
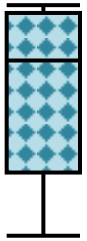
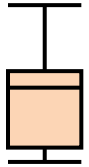
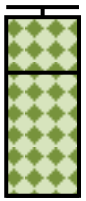
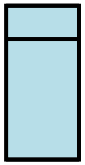
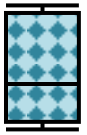
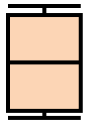
	Low dose (Harro '11)	High dose (historical)
Mod-Sev Diar.	70.9%	77.4%
Any Fever	17.7%	29.0%
Sev Malaise	21.0%	32.3%
Sev Cramps	14.5%	45.2%
Sev Nausea	30.6%	38.7%
Sev Headache	1.6%	32.3%
Any Vomiting	35.5%	35.5%
Early Abx	61.3%	74.2%

Conclusion: Pre-challenge overnight fast yielded comparable rates of moderate-severe diarrhea with 1-2 log lower cfu dose



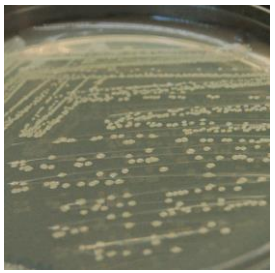
Overnight fast

B7A

Dose (cfu)	10^8	10^9	10^9	10^{10}
Pre-challenge fast	Overnight	90 minutes	Overnight	90 minutes
Mod-Sev diarrhea	42.8%	42.8%	71.4%	71.4%
Early antibiotic	42.8%	42.8%	57.1%	57.1%
Max 24 hr Loose Stool Volume (mL)				
Day 2 B7A shedding				

Dose preparation (traditional)

Day -2

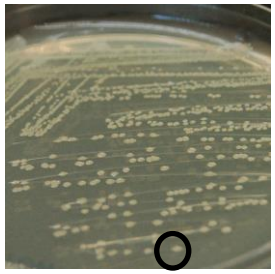


(x6)

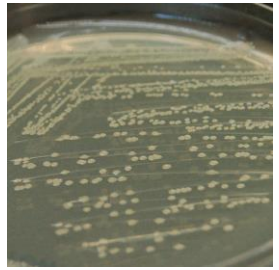
(other media)

37°C

Day -1



Gram stain
Hemagglut.
Colony blots



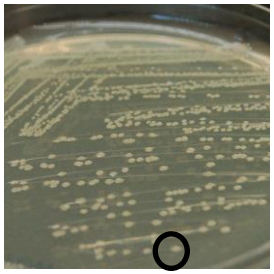
(x2)

(other media)

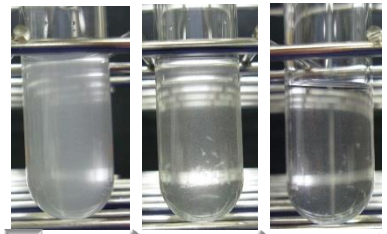
(x14)

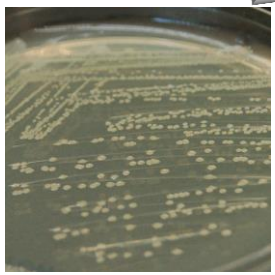
37°C

Day 0



Gram stain
Hemagglut.
Colony blots

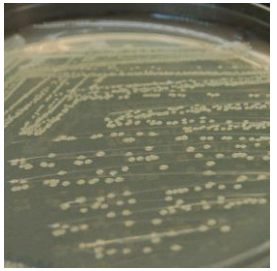




(other media)

37°C

Day 1

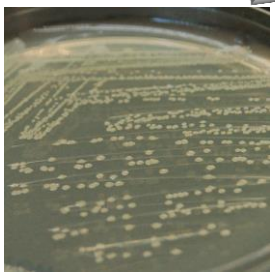


Confirm Dose

Confirm OD_{600}

Dose preparation (refinement)

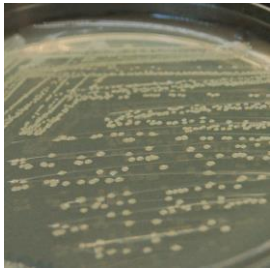
Day 0



37°C

Confirm OD_{600}

Day 1



Confirm Dose

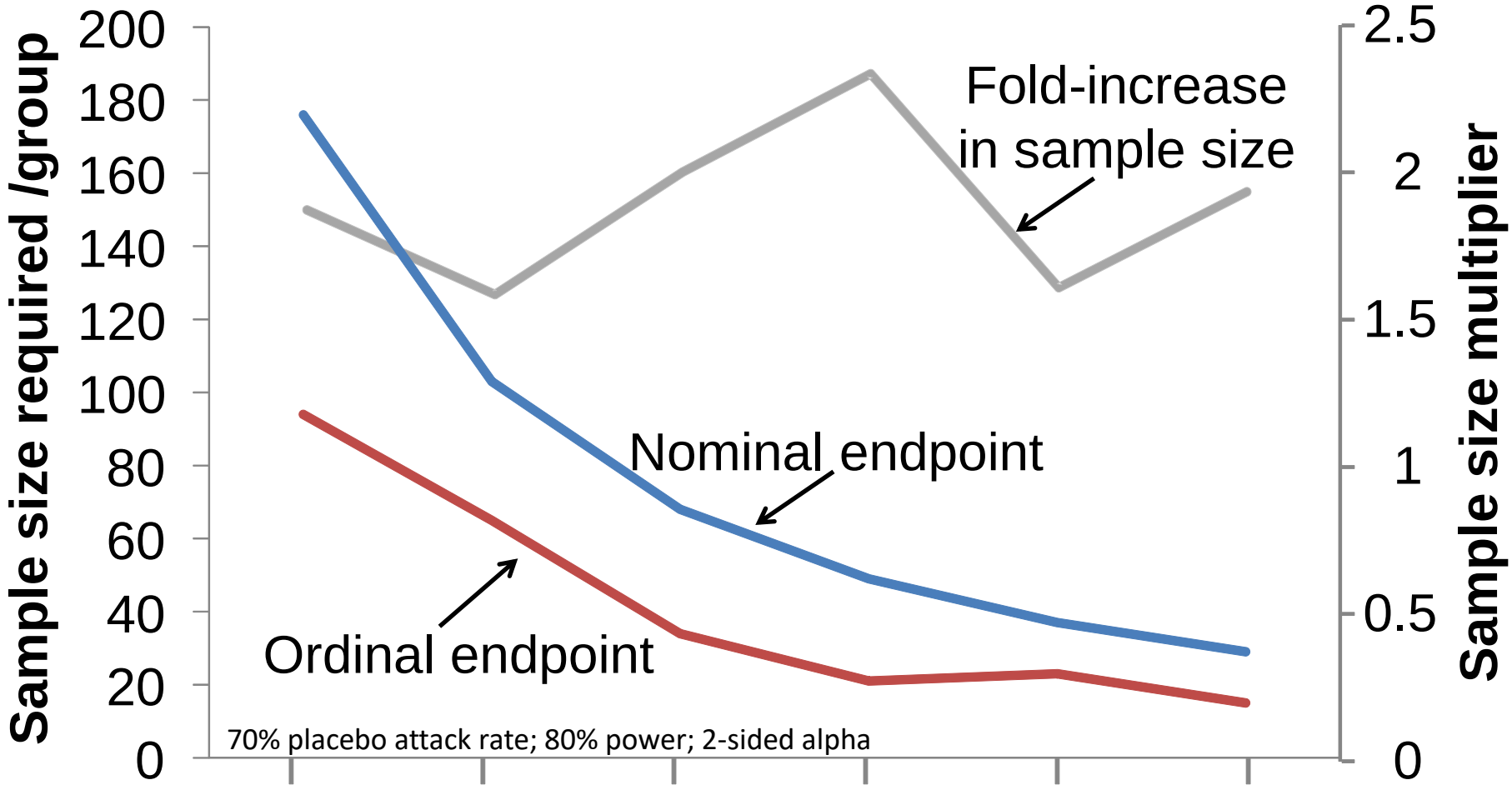
Dose preparation

- Frozen liquid / lyophilized vials
  - H10407 (McArthur *et al* 2017)
    - Frozen GMP material diluted and directly administered (6 subjects)
    - ~10^{10} cfu per vial
    - Mean total loose stool volume: 1.7L
    - Mean total loose stool frequency: 10.2
  - B7A (no clinical data)
    - Lyophilized GMP material
    - Vialed and available for use
    - ~10^{10} cfu per vial

Endpoint refinement: more than stool output

Does NOT meet primary endpoint	1 ls (195 ml); Tmax: 101.1°F; mod malaise & abd cramps
	1 ls (80.1 ml); sev malaise, abd cramps & nausea; vomit (x1)
	1 ls (80.1 ml); sev abd cramps, nausea; vomit (x6); IV rehydration
	3 ls {2 in 24 hrs} (334 ml); mod malaise, abd cramps, nausea; vomit (x1)
	3 ls (380 ml) in 24 hr; severe malaise, abd cramps
Meets primary endpoint	8 ls (749 ml) [in 24 hr: 4 loose stools (473 ml)]; sev malaise, abd cramps, nausea, vomit (x5)
	5 ls (795 ml) [in 24 hr: 4 loose stools (576 ml)]; no other signs/symptoms
	21 ls (6.0 L) [in 24 hr: 13 loose stools (4.6 L)]; Tmax: 101.1°F; sev malaise, abd cramps, nausea, vomit (x3)

Ordinal score vs nominal endpoint



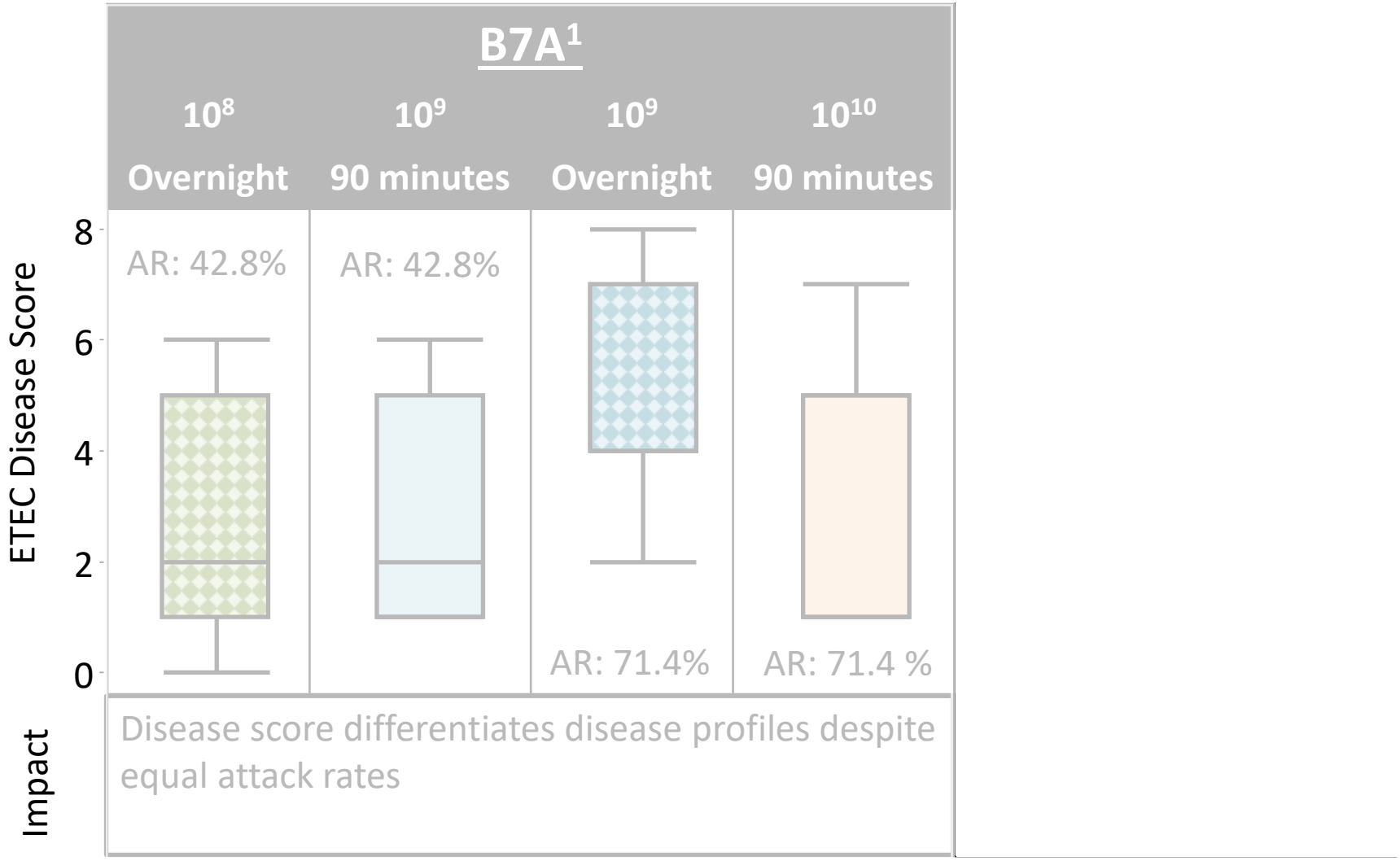
Vacc AR:	55%	50%	45%	40%	35%	30%
Efficacy:	21%	29%	36%	43%	50%	57%

Disease severity score

Parameter	Outcome		Score
Objective signs	>1 episode of vomiting/24 hrs ***OR*** any fever		2
	1 episode of vomiting ***AND*** no fever		1
	No vomiting ***AND*** no fever		0
Subjective symptoms	Moderate-severe lightheadedness ***OR*** Severe: nausea, malaise, headache, abd cramps		2
	Mild lightheadedness ***OR*** mild-mod: nausea, malaise, headache, abd cramps		1
	No 'subjective symptoms'		0
Diarrhea score (max 24 hr loose stools)*	>1000 ml	>12 episodes	4
	>600 to ≤1000 ml	>7 to 12 episodes	3
	>400 to ≤600 ml	>4 to ≤7 episodes	2
	>0 to ≤400 ml	1 to 4 episodes	1
	No loose stools	No loose stools	0

CK Porter et al (2016). "An Evidenced-Based Scale of Disease Severity following Human Challenge with Enteroxigenic *Escherichia coli*." PLoS One **11**(3): e0149358.

Disease severity score

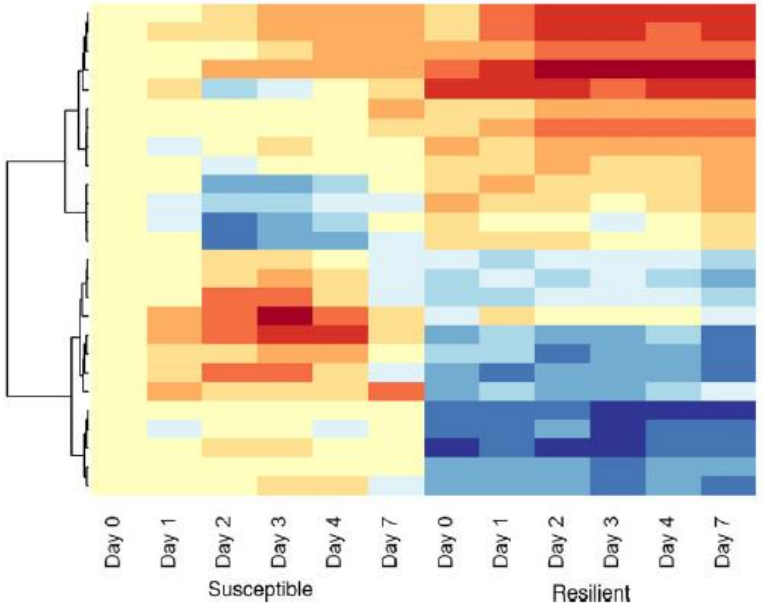


1. ClinicalTrials.gov NCT02773446

2. MJ Darsley (2012). CVI 19(12): 1921-31.

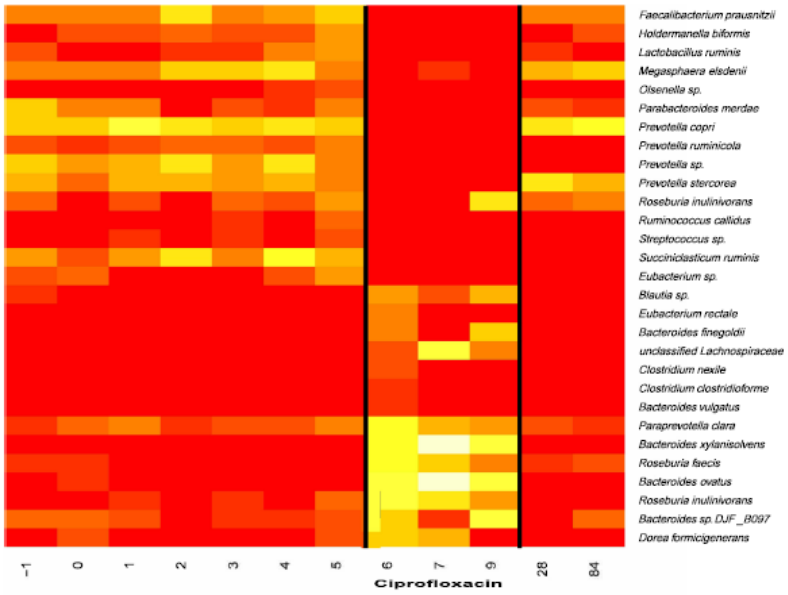
Non-clinical endpoints

Transcriptome



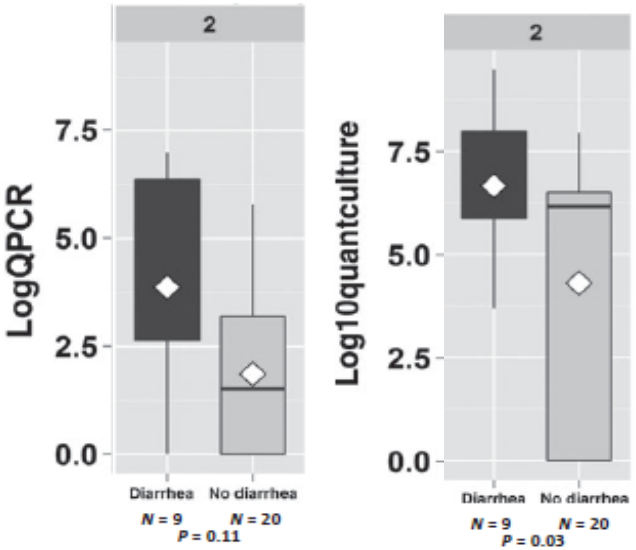
WE Yang et al (2016). J Infect Dis 213(9): 1495-1504.

Microbiome



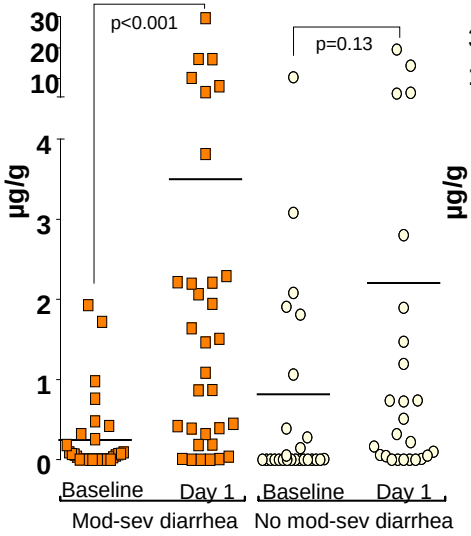
M Pop (2016). BMC Genomics 17(1): 440.

Quantitative shedding

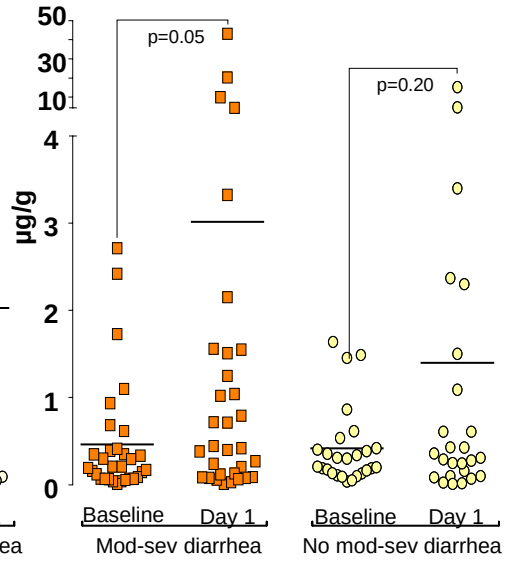


BR Lindsay et al (2014). FEMS Microbiol Lett 352(1): 25-31.

Fecal Calprotectin



Fecal Myeloperoxidase



Unpublished (M. Maciel)

ETEC challenge model constraints

- Limited inpatient facilities
  - Designated ward / facility
  - Experienced personnel
  - Numerous beds (& restrooms)
- Sample size requirements
- Limitations in dose quantification
  - Inoculum to set optical density
  - Pre- / Post-dose plating
  - Perhaps overcome with lyophilized strains
- Inability to quantify the degree of ETEC colonization in the small intestine
- Limited well-characterized strains tested
- Costs

Possible next steps

- Establish new models
  - Alternative colonization factors
  - ST only strains
- Ensure model consistency
  - Overnight fasting
  - Lyophilized lots
- Novel tools to describe strain/host changes
  - Immune profiling and systems biology
  - Challenge organism ‘omics’
  - Microbiome
- Expand application of disease score

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Institute of Research
Soldier Health • World Health