Development of a Low-Dose Challenge Model for Evaluation of Vaccines for Enterotoxigenic *E.coli* (ETEC) in Volunteers

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Background: Enteric Disease Challenge Models

- Variety of ETEC challenge models evaluated since 1970s
- Most extensively studied strain:ETEC H10407 (Serotype 078:K80:H11)
  - >250 subjects challenged
  - Induces reliable AR at doses $\geq 5 \times 10^8$
  - Suitable for vaccine efficacy studies: LT, ST, CFA I

ETEC Challenge Models

- Concern that traditional challenge inoculum artificially high relative to natural exposure
  - May lead to false conclusion that candidate vaccine not protective
  - Other bacterial challenge models typically have lower HD_{50}
- Historically, lowering H10407 inoculum dose has yielded inconsistent AR

Study Objectives

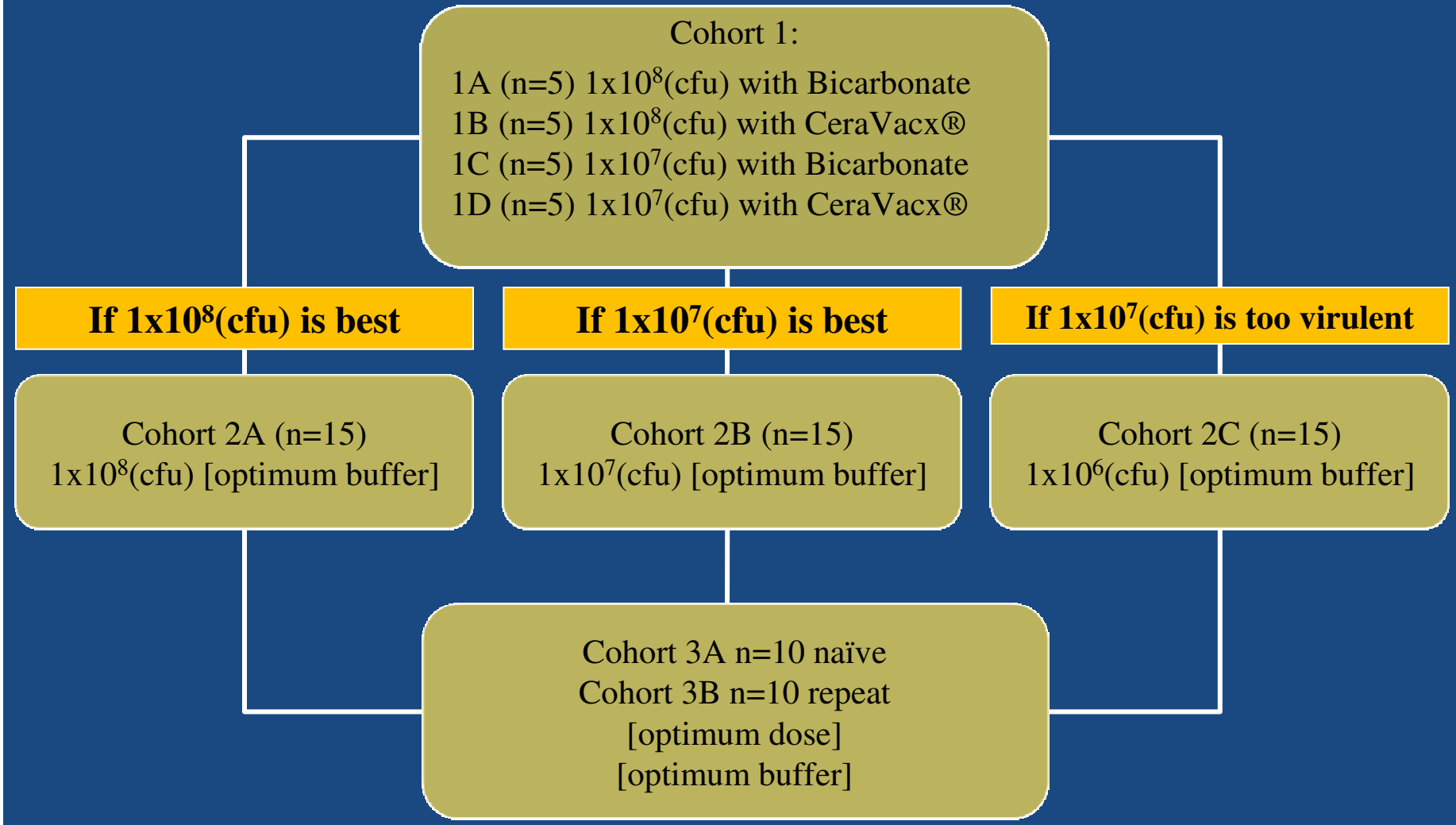
- Identify an H10407 inoculum dose $\leq 10^8$ that will cause diarrhea in 50% or more subjects
- Determine if recent challenge with lower doses or modified delivery approach still protects upon re-challenge
- Measure mucosal and systemic immune responses in naïve and immune subjects using comprehensive assay array
- Determine if mucosal and systemic immune responses predict protection

Study Design Variables

1. Fasting conditions
   - Overnight fast
     - Animal data suggest increased colonization
     - Observational data suggest higher virulence
2. Buffer
   - Bicarbonate buffer
   - Ceravacx®
     - Rice-based bicarbonate/citrate buffer
     - Equivalent gastric acid buffering
     - Rapidly absorbed in glucose-mediated transport
3. Challenge dose

http://www.ceraproductsinc.com/productline/ceravacx.html

Study Design



Methods

- Regulatory approvals obtained January/February 2009
- Recruited healthy volunteers
  - 18-45 yrs
  - No exposure to ETEC, cholera, or LT ≥ 5 years
- Admitted in 3 separate cohorts
  - Cohort 1 February 2009
  - Cohort 2 March 2009
  - Cohort 3 May 2009
- NPO after midnight
- Challenge ~9 hours later
  - 120 mL buffer
  - 30 mL buffer with challenge inoculum

Subject Demographics

		Cohort 1 N=20	Cohort 2 N=15	Cohort 3 N=10*	TOTAL N=45
Male		14 (70%)	11 (73%)	5 (50%)	30 (67%)
Race					
	African American	14 (70%)	11 (73%)	9 (90%)	34 (76%)
	White	4 (20%)	4 (27%)	1 (10%)	9 (20%)
	Other	2 (10%)	0	0	2 (4%)
Age					
	Mean, yrs	30.3	33.6	29.1	31.1
	Range	19-45	19-43	21-41	19-45

* Includes naïve subjects only. Total number of subjects enrolled in Cohort 3 = 20

Medical Monitoring

- Daily history and physical exam
- Collection and grading of all stools
  - Grade 1: Firm, formed (normal)
  - Grade 2: Soft, formed (normal)
  - Grade 3: Viscous, opaque liquid assuming shape of container
  - Grade 4: Watery, non-viscous opaque liquid
  - Grade 5: Clear or translucent watery or mucoid liquid
- Medical management of clinical signs and symptoms
- Independent Medical Monitor

Cohort 1 Results

Cohort 1:

1A (n=5) $1x10^8$(cfu) with Bicarbonate
1B (n=5) $1x10^8$(cfu) with CeraVacx®
1C (n=5) $1x10^7$(cfu) with Bicarbonate
1D (n=5) $1x10^7$(cfu) with CeraVacx®

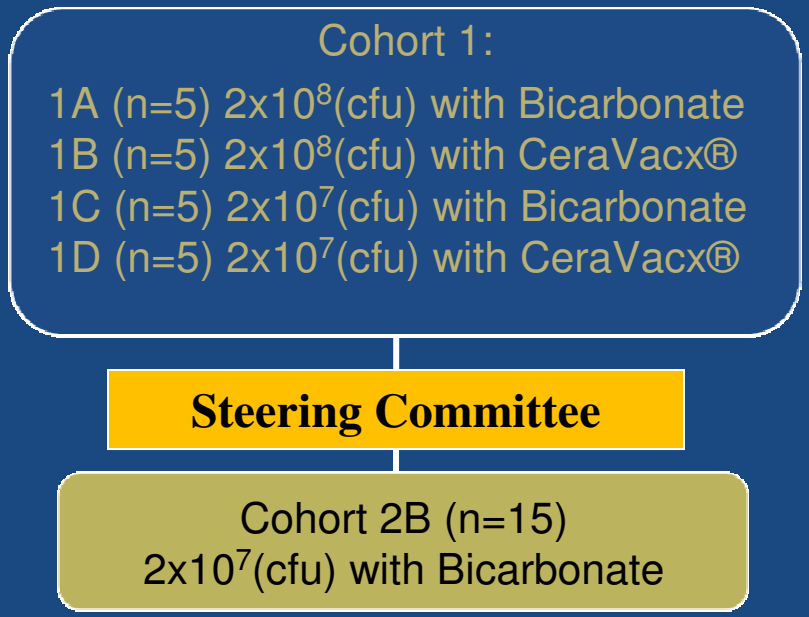
H10407 Challenge Dose	Delivery Vehicle	Diarrhea[1](N)/Challenged (N)	Attack Rate (%)
$2x10^8$ (Cohort 1A)	Bicarbonate	5/5	100
$2x10^8$ (Cohort 1B)	Ceravacx®	4/4*	100
$2x10^7$ (Cohort 1C)	Bicarbonate	4/5	80
$2x10^7$ (Cohort 1D)	Ceravacx®	5/5	100

[1] Diarrhea defined as:
- 1 or more loose stools (≥ Grade 3) of ≥300 grams
- 2 or more loose stools (≥ Grade 3) of ≥200 grams in a 48 hour period

*One subject withdrawn due to noncompliance

Cohort 2

- Rationale
  - AR similar across groups in Cohort 1
- Strategy
  - Lower dose: 10^7cfu
  - Traditional buffer: Bicarbonate



Cohort 2 Results

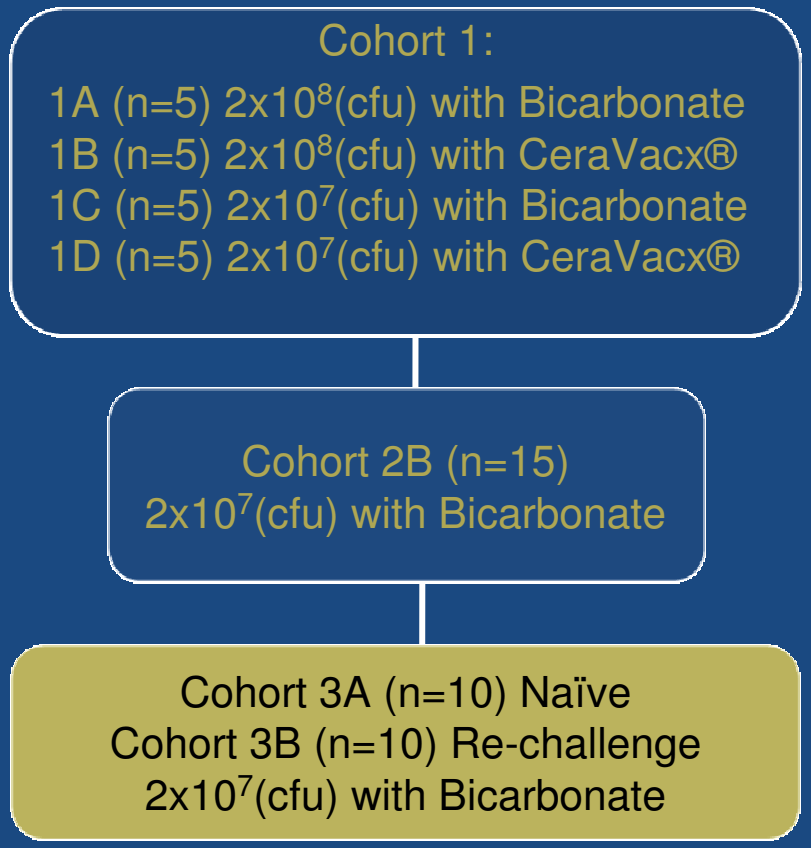
- Confirmed trends observed in Cohort 1
  - Attack Rate ≥50% of challenged subjects
  - Disease severity comparable to higher dose challenge

H10407 Dose	Buffer	Severity of Diarrhea[1]		
		None	Mild	Mod-Severe[2]
$2x10^7$	Bicarbonate	4	0	11 (73%)

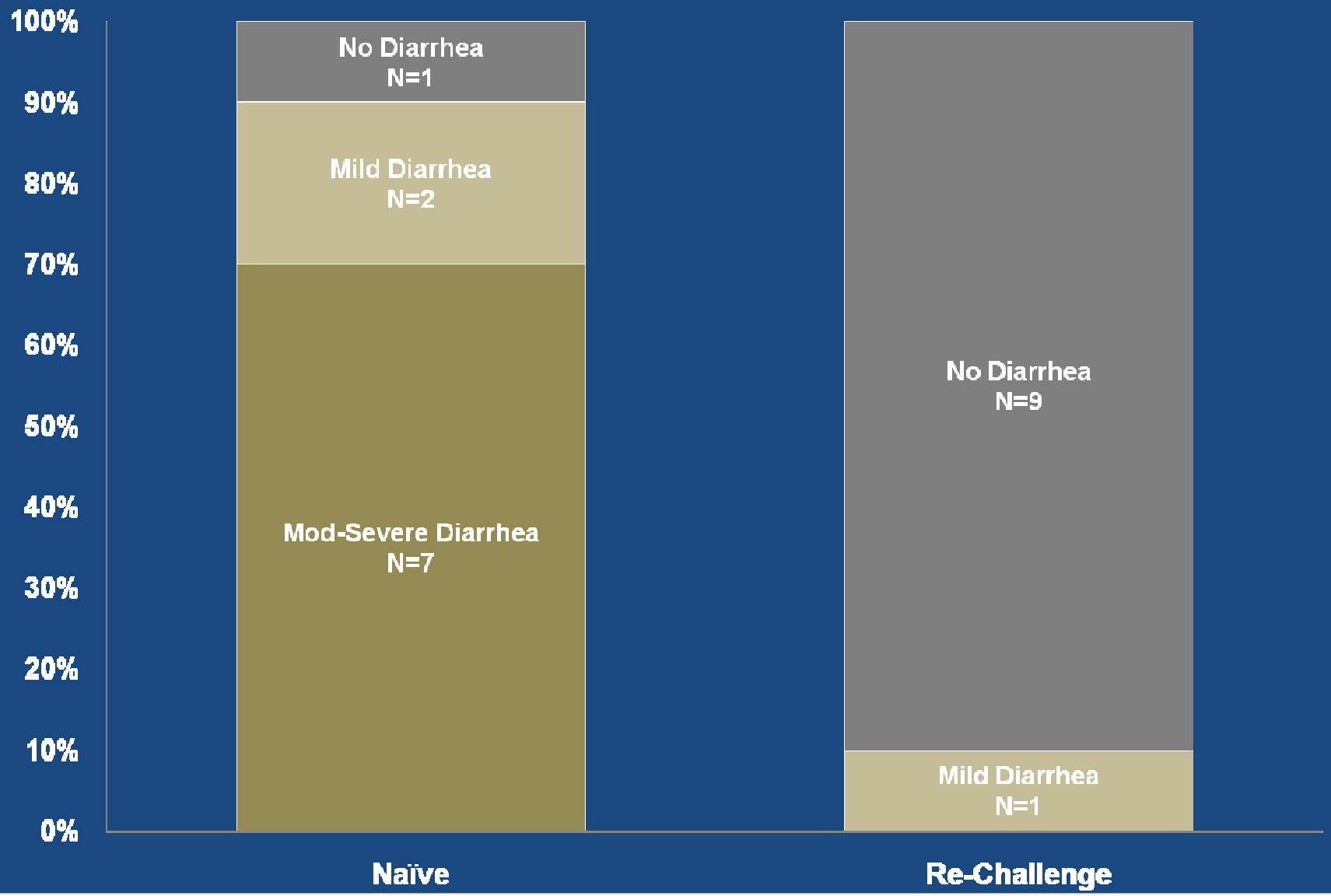
[1] Diarrhea defined as:
- 1 or more loose stools (≥ Grade 3) of ≥300 grams
- 2 or more loose stools (≥ Grade 3) of ≥200 grams in a 48 hour period

[2]Classification based on peak stool number or weight in a 24 hour period
- Moderate: 4-5 stools/24 hrs **or** 401-800 grams/24 hrs
- Severe: ≥ 6 stools/24 hrs **or** >800 grams/24 hrs

Cohort 3 Results



Cohort 3 Results



Combined Outcomes for Subjects Challenged using H10407 Inoculum

Dose (Cohort)	Buffer	Diarrhea[1]			Average Incubation	Early Rx	IV
		None	Mild	Mod-Sev[2]			
$2x10^7$ (Cohort 1)	Bicarbonate N=5	1	0	4 (80%)	43 hrs	3 (60%)	1 (20%)
$2x10^7$ (Cohort 1)	CeraVacx® N=5	0	0	5 (100%)	64 hrs	3 (60%)	1 (20%)
$2x10^7$ (Cohort 2)	Bicarbonate N=15	4	0	11 (73%)	52 hrs	10 (67%)	2 (13%)
$2x10^7$ (Cohort 3)	Bicarbonate N=10	1	2	7 (70%)	57 hrs	5 (50%)	4 (40%)
$2x10^7$ (TOTAL)	**N=35**	**6**	**2**	**27 (77%)**	**54 hrs**	**21 (60%)**	**8 (23%)**

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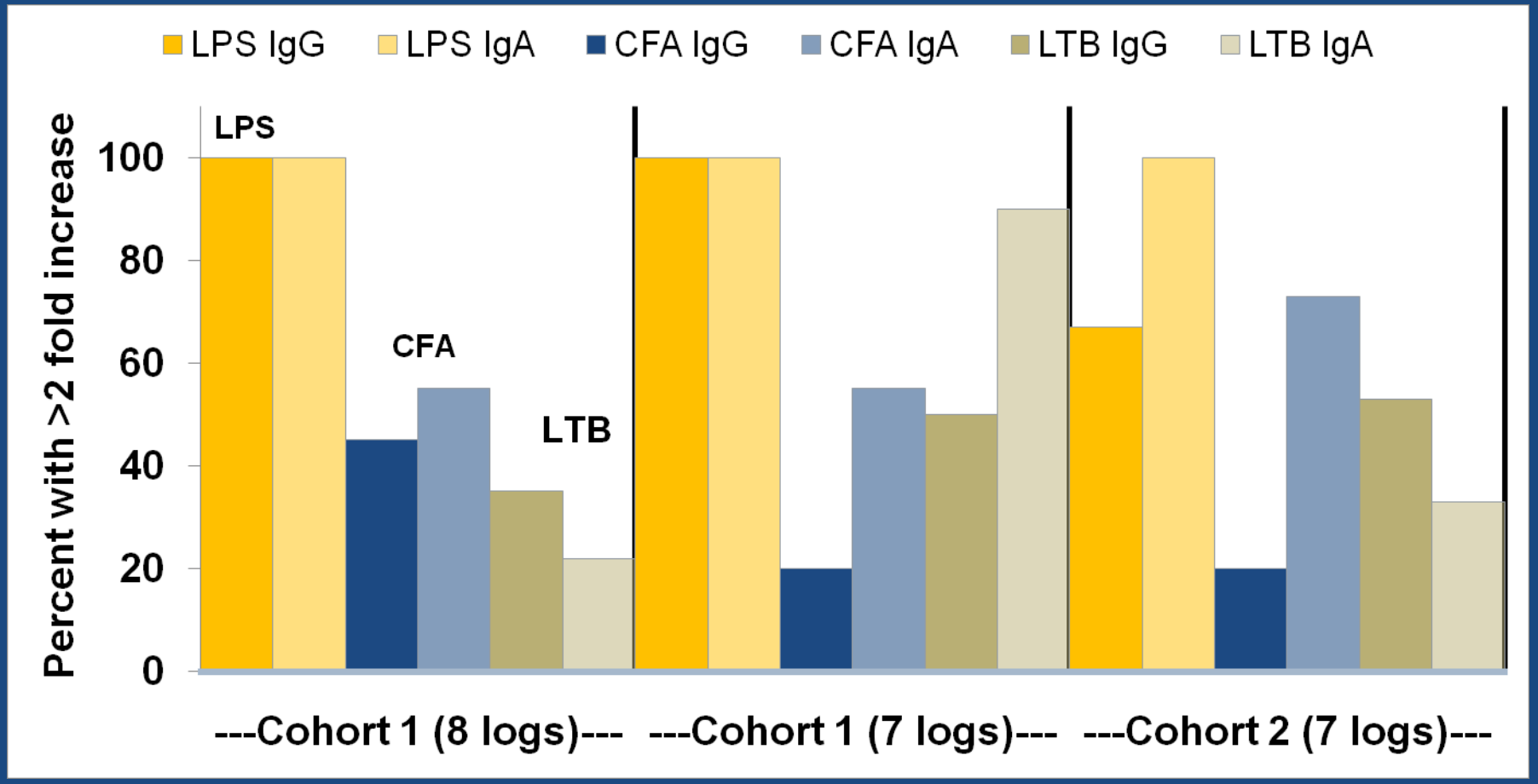
Challenge Strain Shedding

	Number of subjects	Shedding	GeoMean Max. Concentration
Cohort 1*	19	100%	$1x10^8$
Cohort 2	15	100%	$1x10^8$
Cohort 3 (first challenge)	10	90%	$1x10^8$
Cohort 3 (second challenge)	10	90%	$3x10^6$

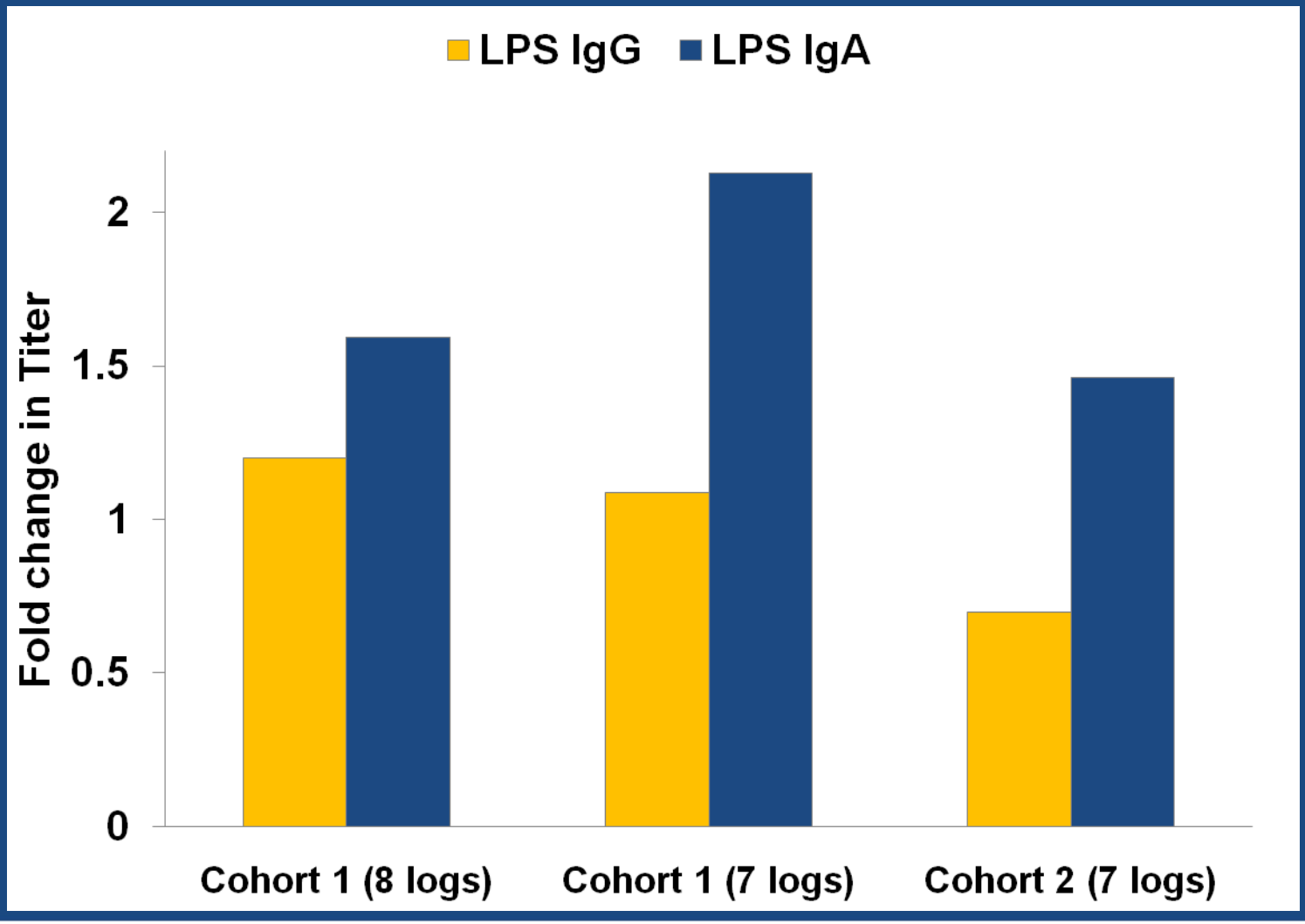
*** No difference in excretion pattern between subgroups of cohort 1**

Seroconversion Rates to H10407 virulence antigens following challenge



Nearly all respond to LPS, fewer to CFA and LTB

Increase in Serum GMT anti-LPS Titers on Day 10 Following Challenge with H10407 (log_{10})



Responses to CFA and LTB

- Late-Breaker Poster for Details
- Serum responses to CFA and LTB were infrequent and low in magnitude
- ALS responses were common and higher, reflecting intestinal immune responses.
- Peak ALS responses were generally on day 7

Summary

- Combined data validate that ETEC H10407 10^7cfu with overnight fast induces:
  - Longer incubation period
  - Reproducible AR $\geq$75%
  - Similar disease severity as higher dose models
- Change in fasting conditions does not alter induction of protective immunity
- Homologous protection confirmed with lower dose model
- Re-challenge data provide opportunity to further explore antigenic determinants of immunity
- Very high and consistent serological responses to LPS, less vigorous responses to CFA and LTB

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