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Development and Clinical Evaluation of an Hyperimmune Bovine Colostrum (HBC) for the Prevention of Campylobacteriosis

Frédéric Poly, Ph.D.

Disclaimer

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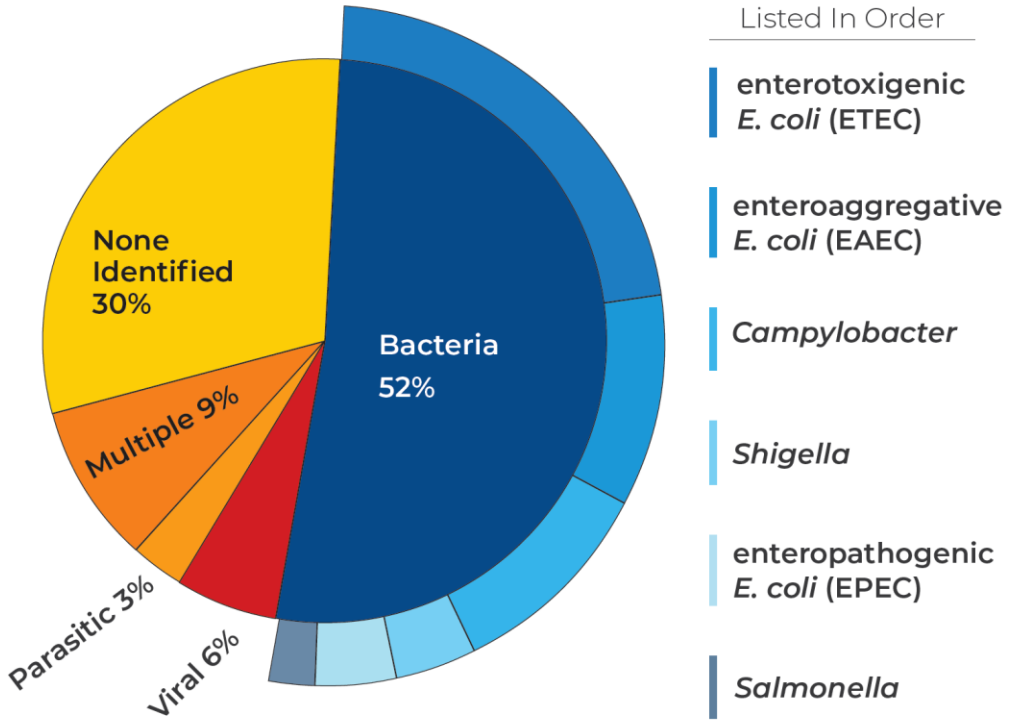
The study protocol CIR360/00026228 was approved by the Johns Hopkins Institutional Review Board in compliance with all applicable federal regulations governing the protection of human subjects. This work was funded by MIDRP (Navy Work unit 6000.RAD1.DA3.A0308).

Introduction

Why the US military is concerned by diarrhea?



Listed In Order

- enterotoxigenic *E. coli* (ETEC)
- enteroaggregative *E. coli* (EAEC)
- *Campylobacter*
- *Shigella*
- enteropathogenic *E. coli* (EPEC)
- *Salmonella*

Operation Iraqi Freedom (OIF)-----Operation Enduring Freedom (OEF)

Diarrhea ranked 1st among 57 infectious disease threats by the 2019 Military Infectious Disease Research Program's Infectious Disease Threat Prioritization Panel based on its impact to readiness.

Bacterial pathogens are the predominant risk, thought to account for the majority of traveler's diarrhea.

76% of Soldiers in OIF and OEF experienced traveler's diarrhea early in their deployment.

The threat of diarrhea will only grow as the effectiveness of antibiotics continues to diminish.

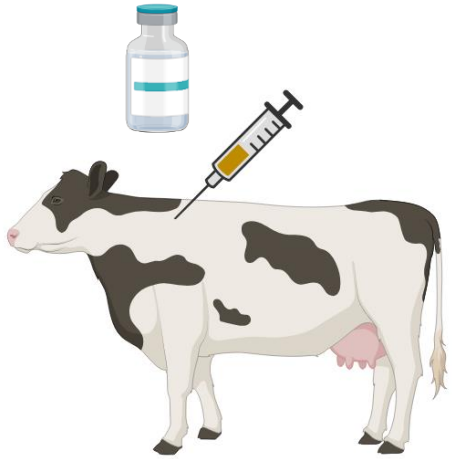
Olson et al. "Tropical Diseases, Travel Medicine and Vaccines, 2019, 5:1-15 Page 3

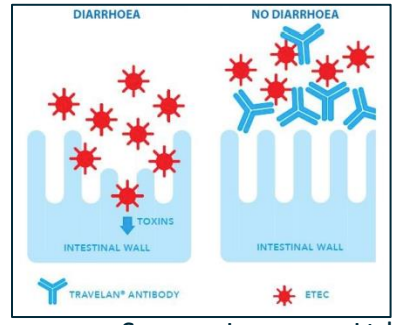
Bovine colostrum

- Colostrum is the first milk secretion of cows after calving
- Contains immunoglobulins, lactoferrin, lysozyme,
- growth factors
- Cow produces approximately 5 to 10 L of colostrum per milking
- Bovine colostrum (i.e. Spray dried) consumption is being encouraged as a nutritional supplement

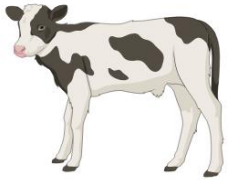
Hyperimmune Bovine Colostrum (HBC)

- **Production of Hyperimmune Bovine Colostrum (HBC) made from cows vaccinated with an antigen of choice**
- **Demonstrated protective efficacy against bacterially-diarrhea-causing pathogens**

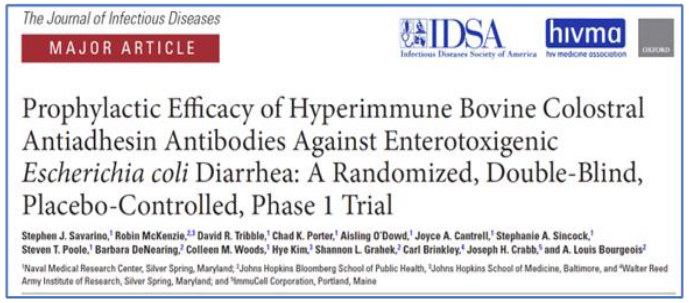




Source: Immuron Ltd



Pregnant cow



The Journal of Infectious Diseases

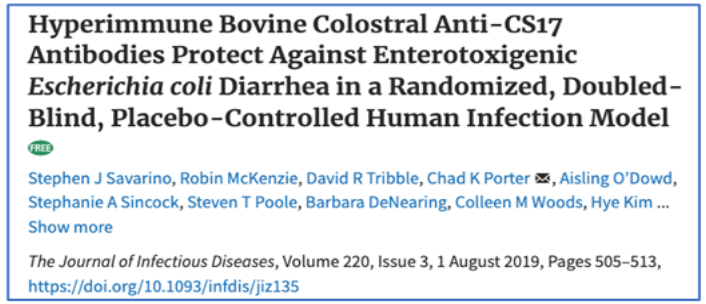
MAJOR ARTICLE

IDSA Infectious Diseases Society of America · hivma hiv medicine association

Prophylactic Efficacy of Hyperimmune Bovine Colostral Antiadhesin Antibodies Against Enterotoxigenic *Escherichia coli* Diarrhea: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Trial

Stephen J. Savarino,[1] Robin McKenzie,[2,3] David R. Tribble,[1] Chad K. Porter,[1] Aisling O'Dowd,[1] Joyce A. Cantrell,[1] Stephanie A. Sincock,[1] Steven T. Poole,[1] Barbara DeNearing,[2] Colleen M. Woods,[1] Hye Kim,[3] Shannon L. Grahek,[2] Carl Brinkley,[4] Joseph H. Crabb,[5] and A. Louis Bourgeois[2]

[1]Naval Medical Research Center, Silver Spring, Maryland; [2]Johns Hopkins Bloomberg School of Public Health, [3]Johns Hopkins School of Medicine, Baltimore, and [4]Walter Reed Army Institute of Research, Silver Spring, Maryland; and [5]ImmuCell Corporation, Portland, Maine



Hyperimmune Bovine Colostral Anti-CS17 Antibodies Protect Against Enterotoxigenic *Escherichia coli* Diarrhea in a Randomized, Doubled-Blind, Placebo-Controlled Human Infection Model

FREE

Stephen J Savarino, Robin McKenzie, David R Tribble, Chad K Porter ✉, Aisling O'Dowd, Stephanie A Sincock, Steven T Poole, Barbara DeNearing, Colleen M Woods, Hye Kim ...

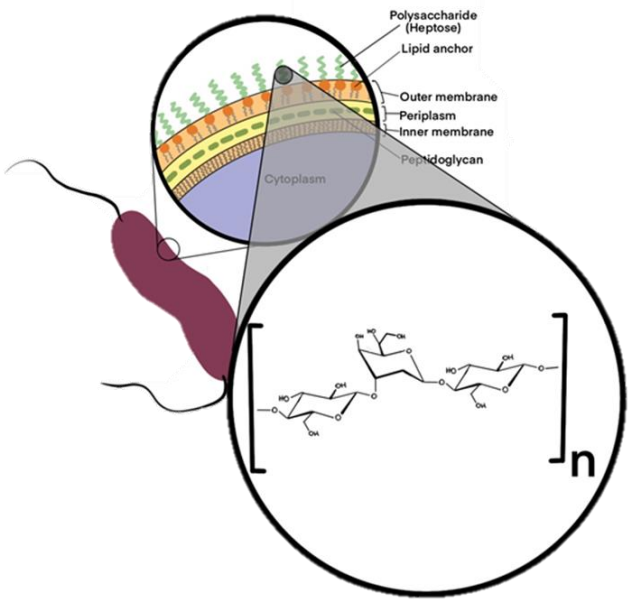
Show more

The Journal of Infectious Diseases, Volume 220, Issue 3, 1 August 2019, Pages 505–513, https://doi.org/10.1093/infdis/jiz135

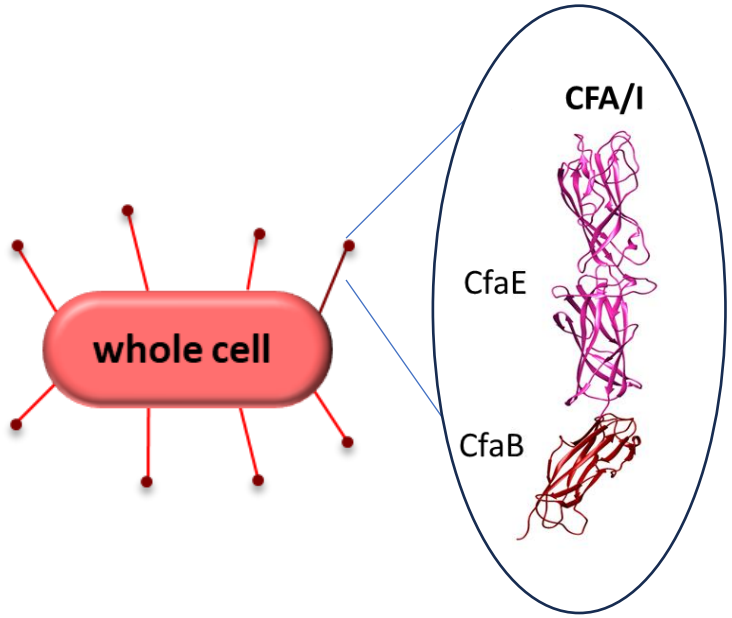
Step 1:
Vaccine production

Vaccine components

- Leverage departmental vaccine research
- Developed a bi-pathogen vaccine:
  - Recombinant fusion protein (CfaE) and pilin (CfaB) of ETEC Colonization Factor I (CFA/I)
  - *C. jejuni* HS23/36 capsule polysaccharide



C. jejuni capsule



ETEC Colonizing Factor

C. jejuni capsule conjugated to ETEC CF (NMRC)

- **Reductive amination of the HS23/36 *C. jejuni* capsule (81-176 strain)**
- **Conjugation to NH_2 residues of Lysine**

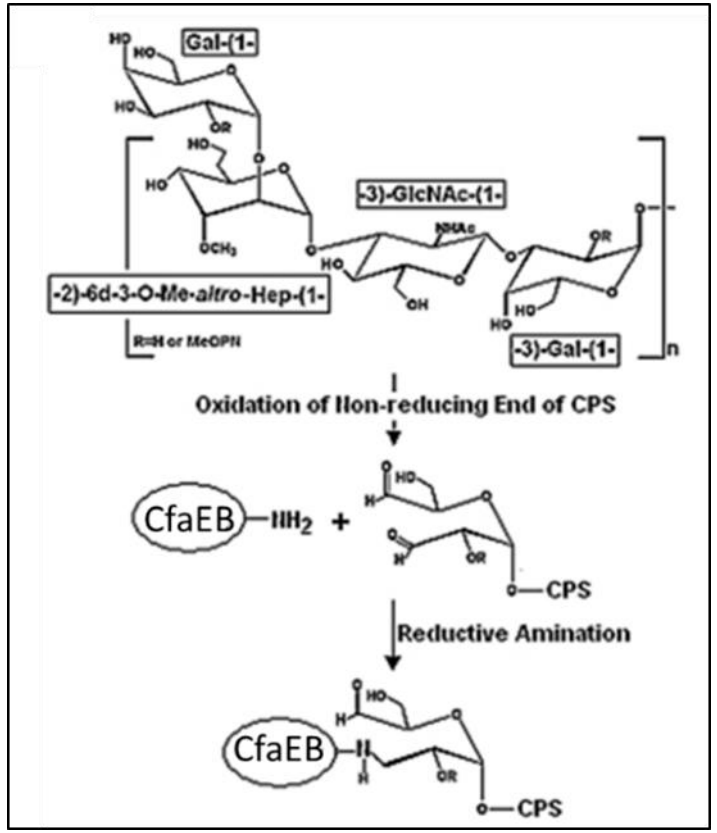
Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Evaluation of a conjugate vaccine platform against enterotoxigenic *Escherichia coli* (ETEC), *Campylobacter jejuni* and *Shigella*

Renee M. Laird [a,b,*], Zuchao Ma [c], Nelum Dorabawila [a,b,1], Brittany Pequegnat [c], Eman Omari [c], Yang Liu [a,b], Alexander C. Maue [a,b,2], Steven T. Poole [a,b], Milton Maciel [a,b], Kavyashree Satish [a,b], Christina L. Gariepy [a,b], Nina M. Schumack [a,b], Annette L. McVeigh [a,b], Frédéric Poly [b], Cheryl P. Ewing [a,b], Michael G. Prouty [b], Mario A. Monteiro [c], Stephen J. Savarino [b,3], Patricia Guerry [b,*]



Adapted from Monteiro et al., 2009

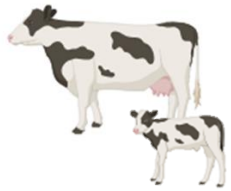
Step 2:
HBC production
CampETEC product

Vaccination

3 months
prior to calving

2 months
prior to calving

1 month
prior to calving



calving

27 liters

- Collaboration with Immuron Limited, Australia
- Vaccination performed at CSIRO, Armidale, NSW Australia
- Vaccine: Dose 1&2: 1 mg CPS-CfaEB + Adjuvant
  Dose 3: 2 mg CPS-CfaEB + Adjuvant

CampETEC Processing

- Performed at CSIRO, Werribee, VIC Australia
- Generated 1.2 kg dried of material= CampETEC

Colostrum Frozen Storage

Colostrum Tempering, Thawing & Blending

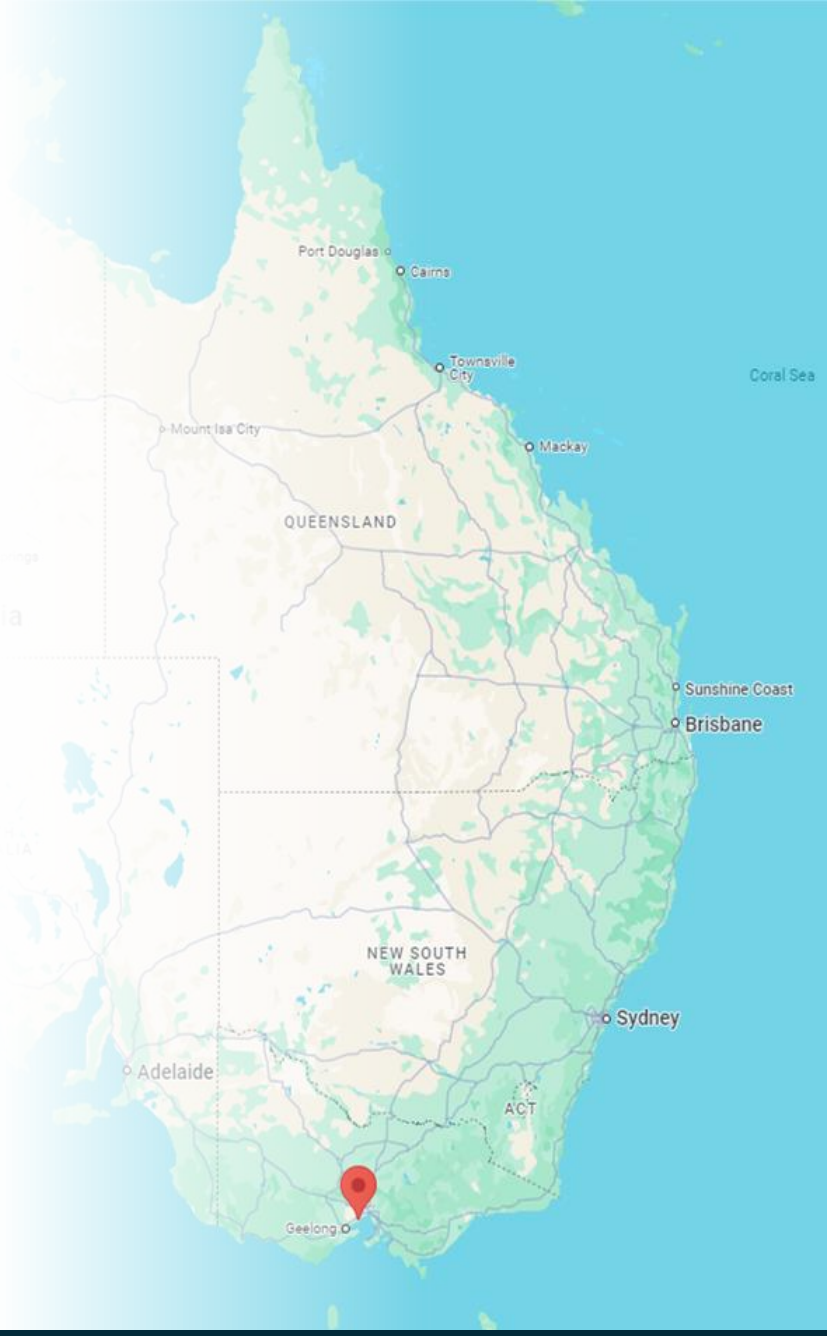
Filter, pH adjustment & Skim Dilution

Pasteurization

De-fatting by centrifugation

Ultrafiltration

Spray Dry



CampETEC Release Testing

Appearance:

- White to pale yellow powder

Microbial Testing:

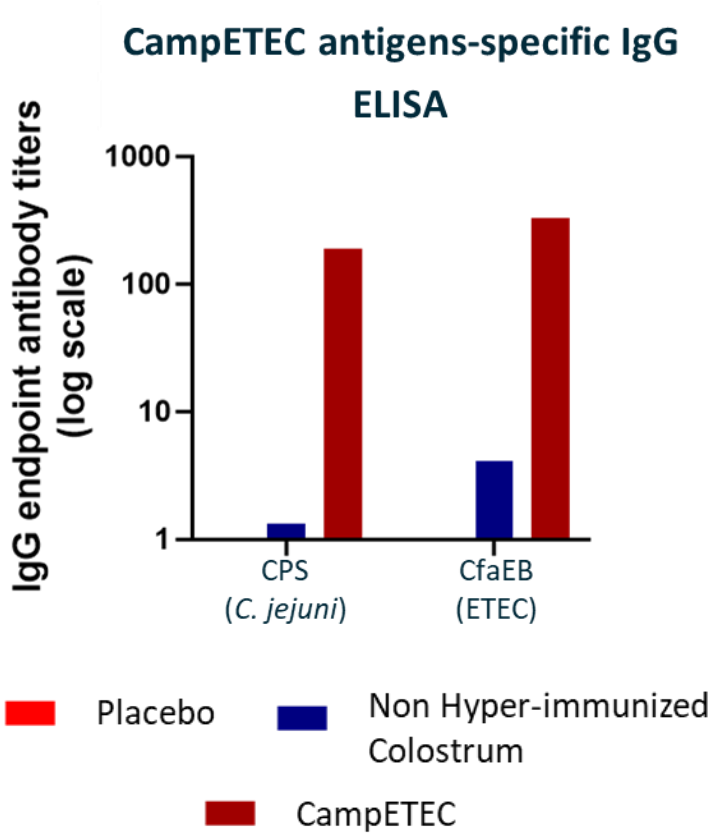
- Total aerobic microbial count 2.3 x 10^3 cfu/g
- *E. coli,* Salmonella, Listeria, Streptococcus (Group A & B) <100 cfu/g

Chemical testing:

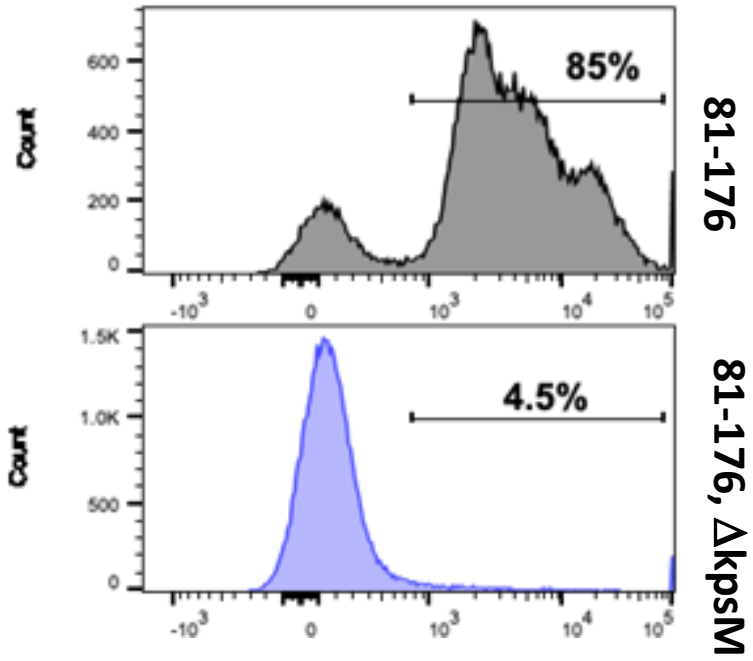
- IgG (total) 54.4%
- Water Content 8%
- Total Protein 86%
- Bacterial Endotoxins 0.152 EU/mg

CampETEC Characterization

- Total IgG content
- IgG1, IgG2 content
- IgA content
- IgM content
- Growth factors
- Cytokines

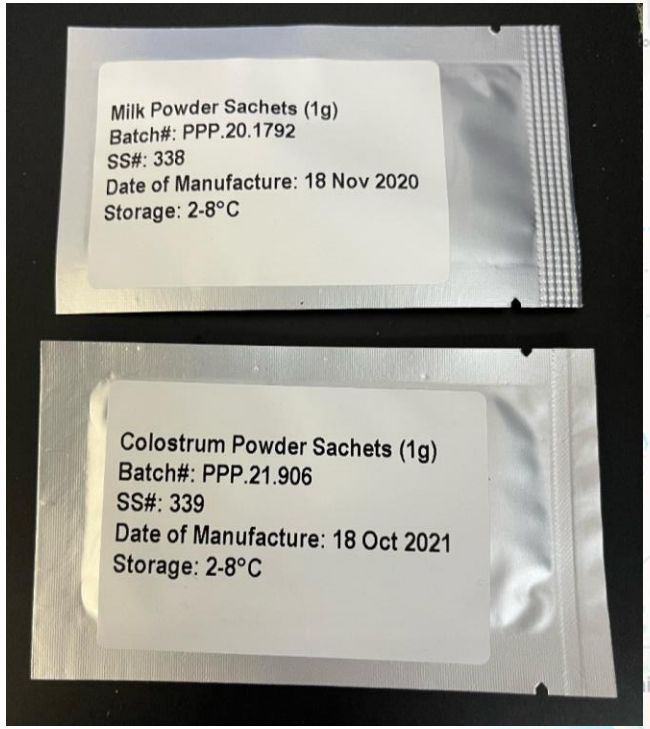


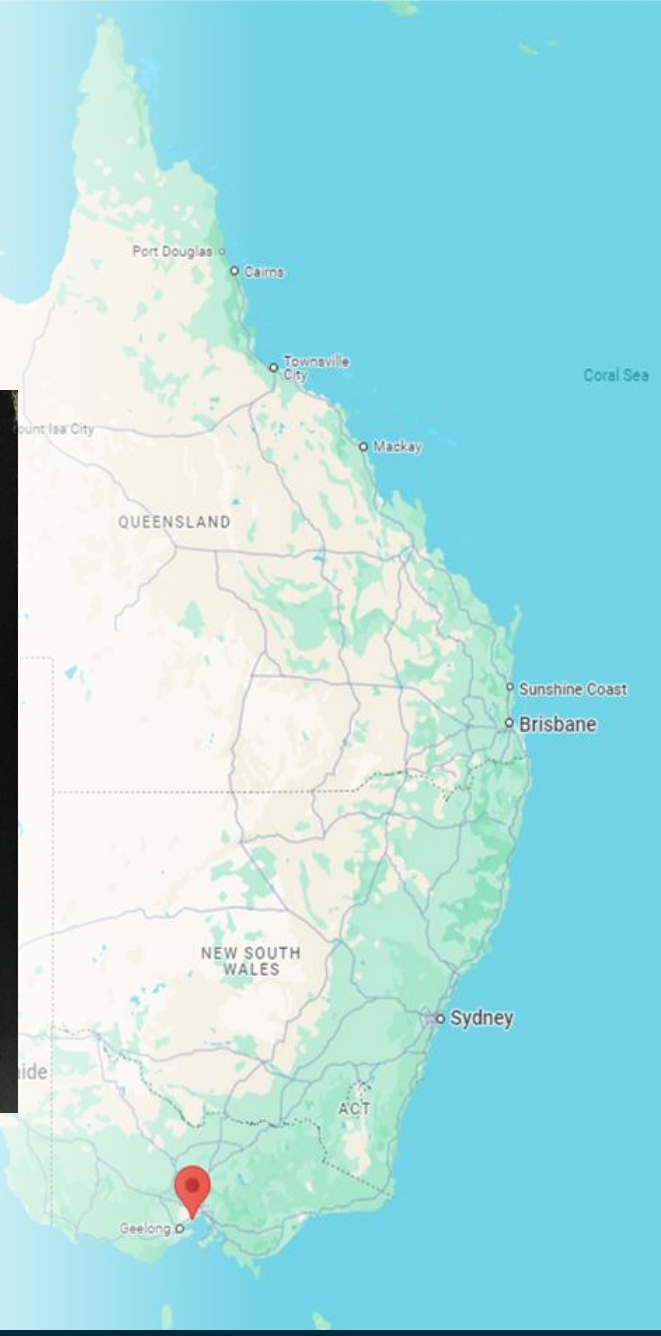
Flow binding assay



CampETEC Fill/Finish

- Contracted PCI Pharma Services, Melbourne, VIC Australia
- Powder and placebo tested, packaged and labeled CampETEC





Step 3:
CampETEC Clinical Evaluation

Randomized, double-blind, placebo controlled human *C. jejuni* infection model

- **Principal Investigator: Kawsar R Talaat, MD, Johns Hopkins Center for Immunization Research**
- **Sponsor- Johns Hopkins Bloomberg School of Public Health**
- **ClinicalTrials.gov Identifier NCT06122870**
- **In patient phase performed in December 2023**

Johns Hopkins Bayview Medical Center

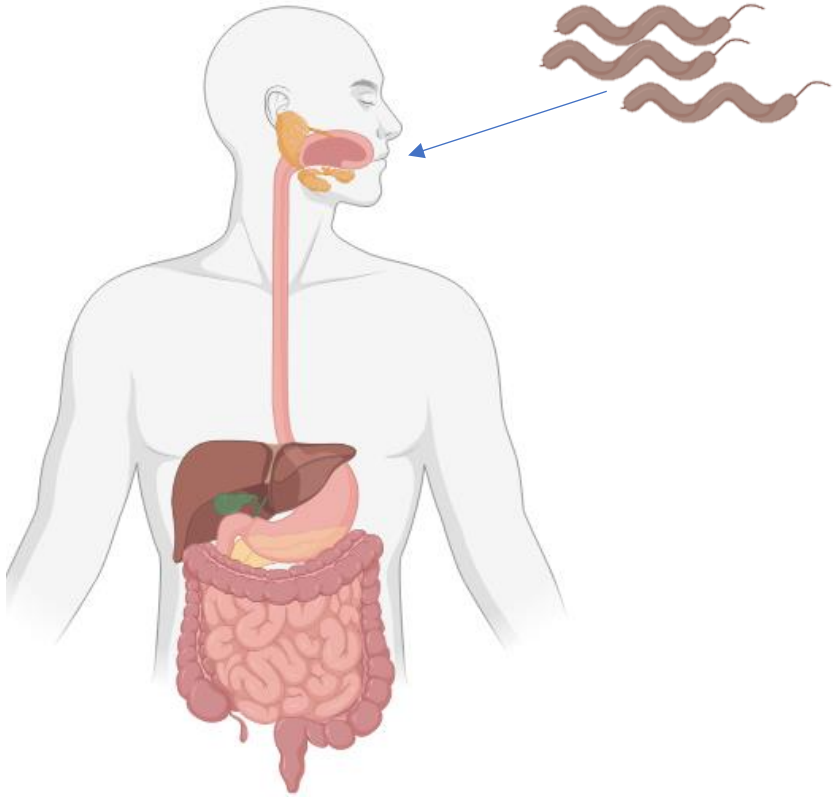
C. jejuni CG8421 challenge model

- **Established in 2007 at the University of Vermont**
- **No ganglioside mimicry**
- **Inoculum dose of $5x10^5$ CFU**
- **Median time to diarrhea onset is ~ 72.3 h**
- **No major safety concerns but recrudescence can occur**

Campylobacter jejuni Strain CG8421: A Refined Model for the Study of Campylobacteriosis and Evaluation of *Campylobacter* Vaccines in Human Subjects

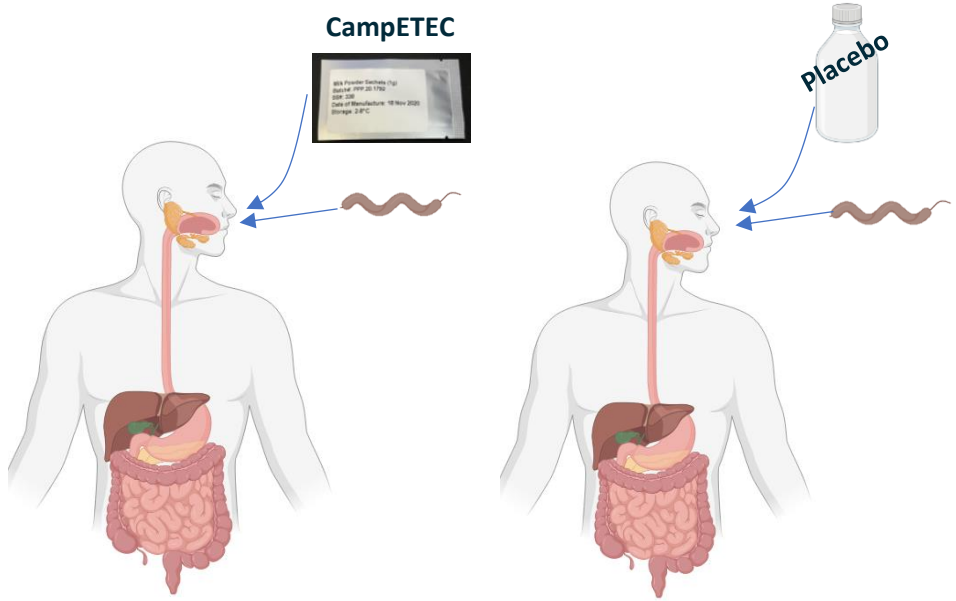
David R. Tribble,[1,2] Shahida Baqar,[1,2] Marya P. Carmolli,[3] Chad Porter,[1] Kristen K. Pierce,[3] Katrin Sadigh,[3] Patricia Guerry,[1] Catherine J. Larsson,[3] David Rockabrand,[1] Cassandra H. Ventone,[3] Frederic Poly,[1] Caroline E. Lyon,[3] Sandra Dakdouk,[1] Ann Fingar,[3] Theron Gilliland, Jr,[1] Patrick Daunais,[3] Erika Jones,[1] Stacia Rymarchyk,[3] Christopher Huston,[3] Michael Darsley,[4] and Beth D. Kirkpatrick[3]

[1]Department of Enteric Diseases, Naval Medical Research Center, and [2]Department of Preventive Medicine and Biometrics, Uniformed Services



Randomized, double-blind, placebo controlled human *C. jejuni* infection model

- 27 healthy 18-50 years old volunteers
- All volunteers were challenged with 1.67 x 10e5 colony-forming units of CG8421
- Received 1 gram of CampETEC or Placebo (milk product) 3 times daily, 15 minutes following each meal
- 2 days prior to challenge until day 7 or they meet the criteria for antibiotics



n=13 n=14

Randomized, double-blind, placebo controlled human *C. jejuni* infection model

- **Primary Objectives**
  - **Estimate protective efficacy (PE)** of CampETEC HBC against campylobacteriosis following challenge with ***C. jejuni* strain CG8421**
    - **Moderate diarrhea** (4 to 5 loose/liquid stools or 401-800 grams) **OR**
    - **Severe diarrhea** (≥6 loose/liquid stools or >800 grams) **OR**
    - **Fever** ***(present on at least 2 occasions, at least 20 minutes apart) without diarrhea, plus an associated symptom (nausea, vomiting, abdominal cramps, tenesmus, or gross blood in ≥2 stools); with consideration of potential alternative diagnosis per clinical investigator based on illness time course and associated symptoms***
  - **To assess the safety and tolerability of CampETEC HBC**
- **Secondary Objective**
  - **To assess the ability of CampETEC HBC to prevent or reduce a variety of secondary clinical endpoints**

Clinical Trial preliminary results

- Late August 2024, the final determination of campylobacteriosis was adjudicated

	CampETEC	Placebo	p-value
Campylobacteriosis, n (%)	10/13 (76.9)	12/14 (85.7)	0.6
Fever, n (%)	6/13 (46.2)	5/14 (35.7)	0.7
Diarrhea, n (%)	12/13 (92.3)	13/14 (92.9)	1.0
Mild	1/12 (8.3)	1/13 (7.7)	--
Moderate	5/12 (41.7)	5/13 (38.5)	--
Severe	6/12 (50.0)	7/13 (53.8)	--
Maximum weight (g) loose stool weight in 24 hrs, mean (std dev)	570.9 (302.8)	736.2 (427.8)	0.5
Maximum number of loose stools in 24 hrs, mean (std dev)	5.2 (3.3)	5.6 (3.6)	1.0
Early antibiotics, n (%)	6/13 (46.2)	7/14 (50.0)	1.0

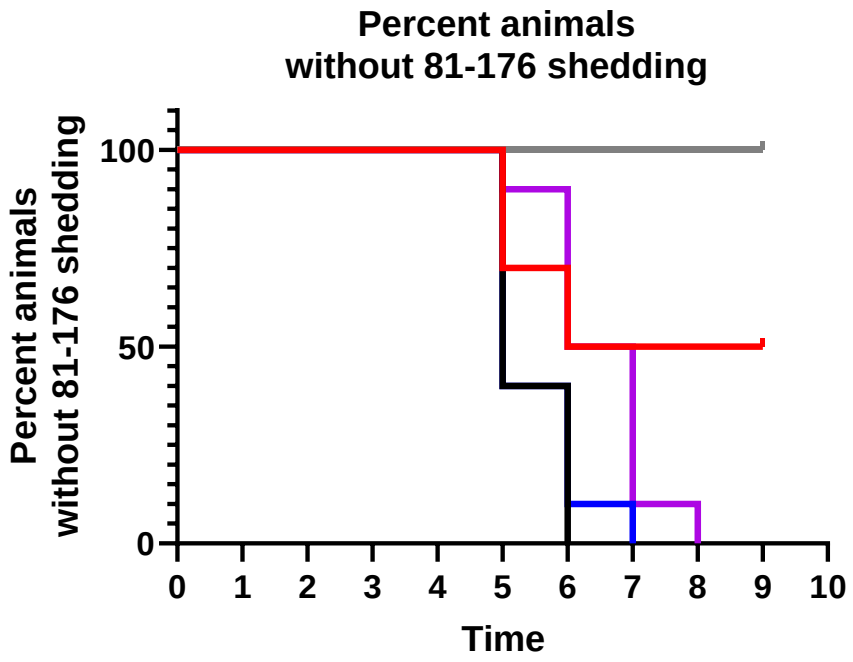
Conclusion & Future direction

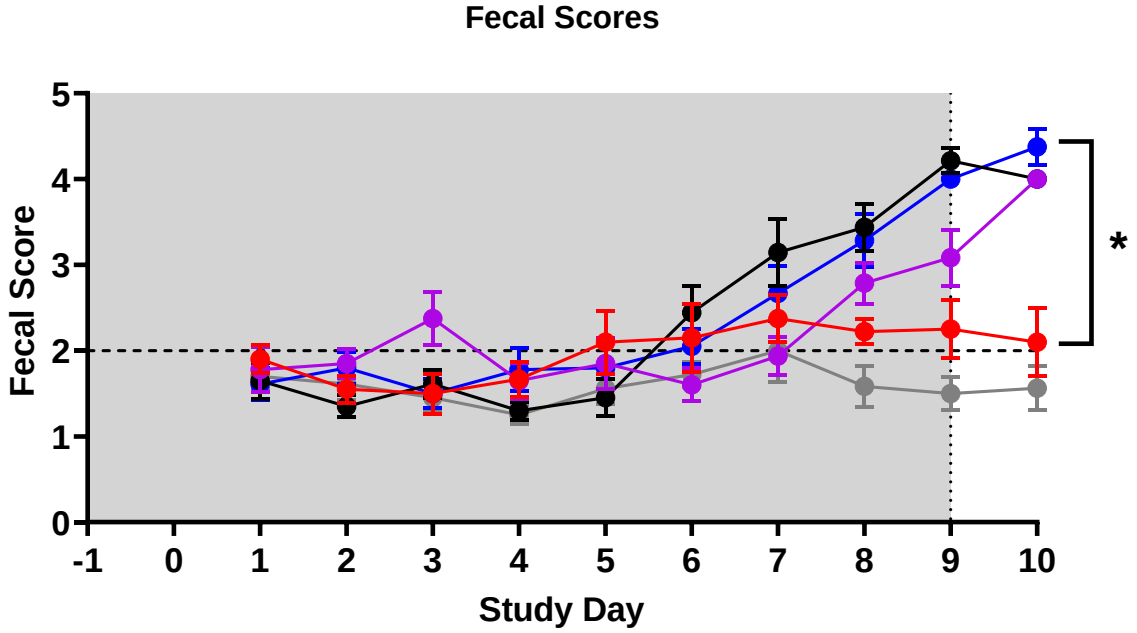
- *C. jejuni* challenge strains is performing well (attack rate of 86%, at 1.7 10^5 CFU)
- CampETEC was well-tolerated
- No moderate or severe adverse events were reported

- CampETEC did not significantly prevent campylobacteriosis
- Serum IgA and IgG measurements are underway

- Regimen dose of CampETEC not enough/ Too many bacteria in the inoculum
- HBC targeting only the polysaccharide is not enough
  - Conjugation to Campylobacter specific proteins
  - Whole cell approach for the development of HBC

Mouse Zn deficient animal model





Sham
No prophylaxis
Bovine colostrum
Campy HBC 1.125 mg
Campy HBC 2.5 mg

Acknowledgments

NMRC/ ORI

- Dr. Renee Laird
- Mrs. Vicky Chapman
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- Ms. Katie Dori
- Mrs. Nina Shoemaker
- Ms. Heather Eggleston
- Mr. Aaron Kim
- Ms. Janelle Kuroiwa
- Mrs. Christina Gariepy

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Immuron

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