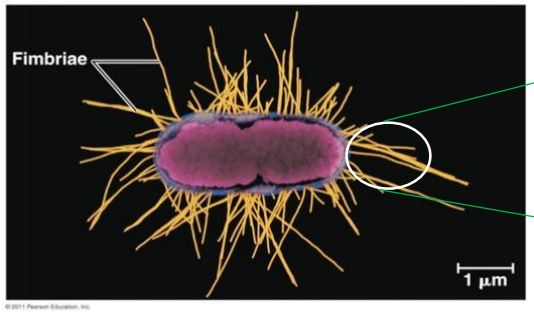
New Strategies in Development of Vaccines against ETEC

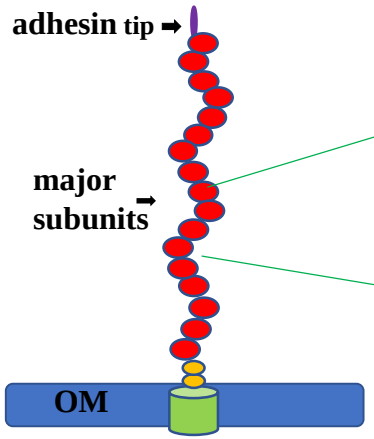
Weiping Zhang

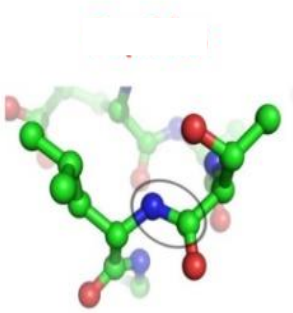
University of Illinois at Urbana-Champaign,
Department of Pathobiology, Illinois, USA

Vaccinology Development and Evolution

organism → organelle → structural subunit → neutralizing epitope

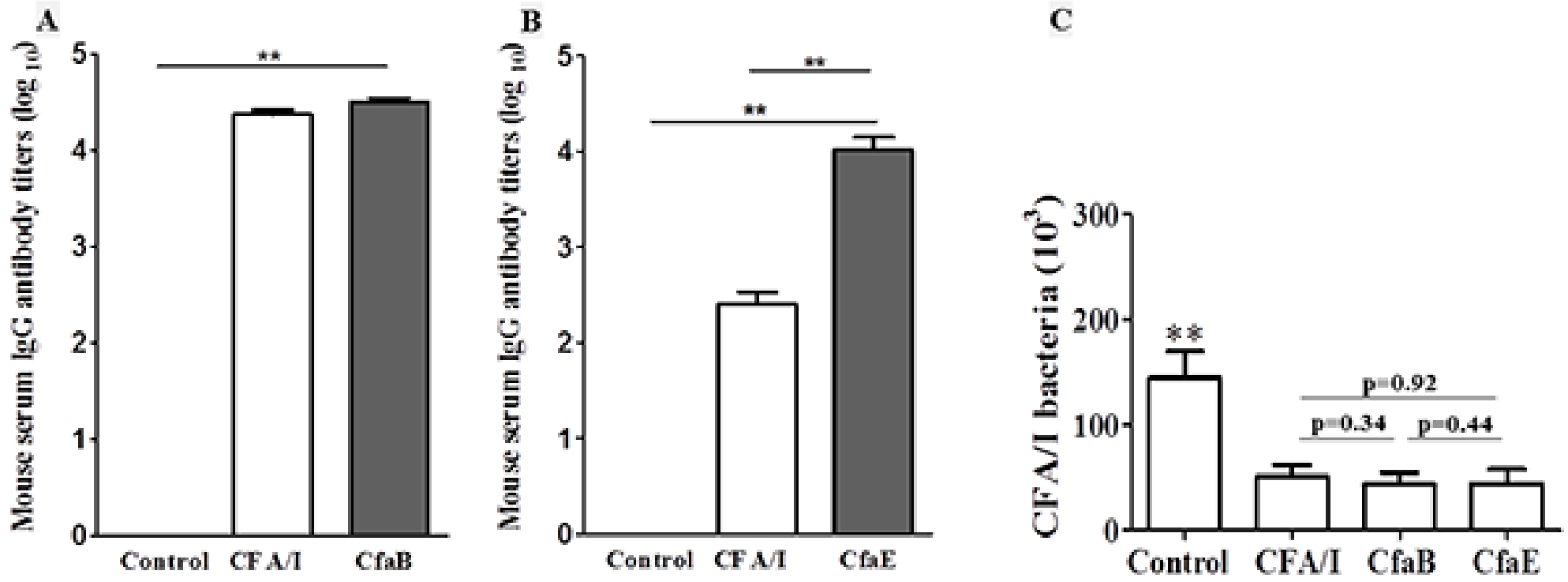






whole-cell vaccine → subunit vaccine → epitope + structural precision vaccine

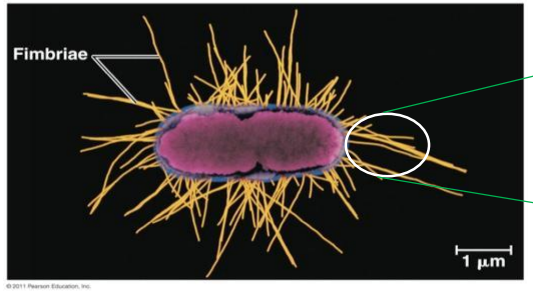
Antibodies to CFA/I fimbriae, major subunit or minor tip subunit are equivalently effective against ETEC adherence

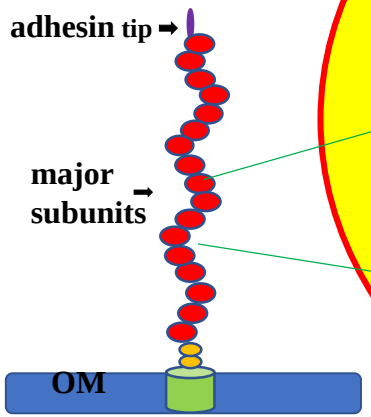


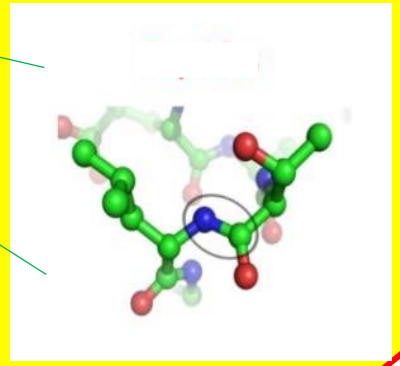
Seo et al., 2019, PLoS ONE, 14(5):e0216076

Vaccinology Development and Evolution

organism → organelle → structural subunit → neutralizing epitope

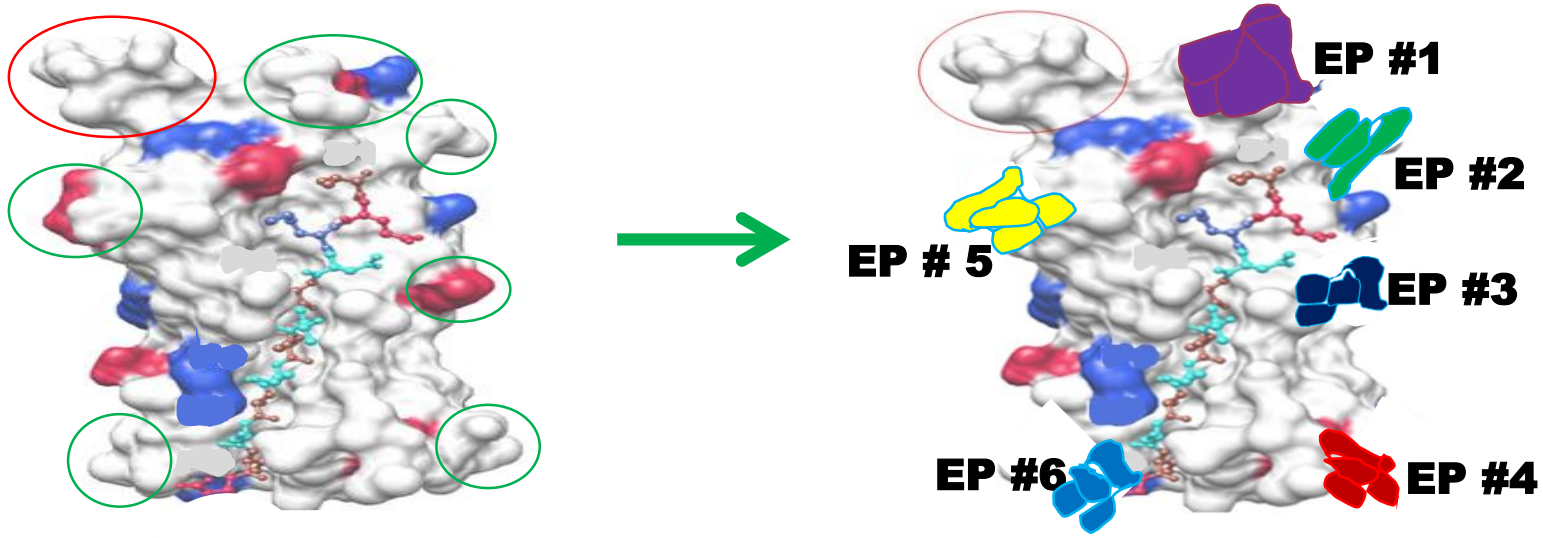






whole-cell vaccine → subunit vaccine → epitope + structural precision vaccine

MEFA (multiepitope fusion antigen) technology - epitope- & structure-based vaccinology



backbone

MEFA

MEFA technology for a multivalent PWD vaccine

Virulence factors of ETEC in pig diarrhea

I. *Fimbrial adhesins*

- **K88 (F4; ab, ac ad)**
- **K99 (F5)**

- **987P (F6)**
- **F41 (F7)**
- **F18 (ab, ac)**

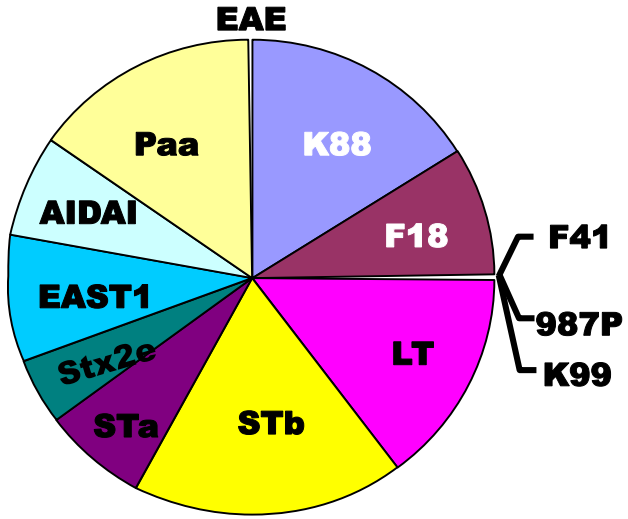
II. *Enterotoxins*

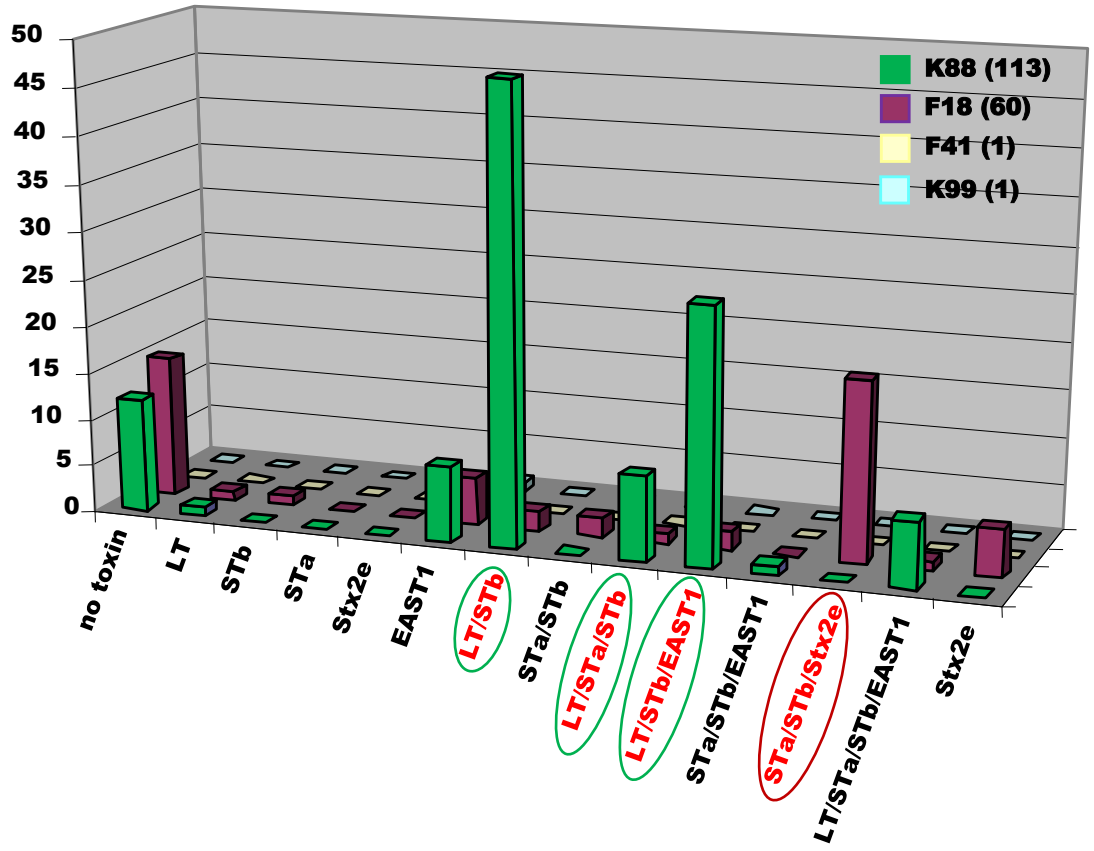
- **heat-labile toxin (LT)**
- **heat-stable toxin type Ia (pSTa)**
- **heat-stable toxin type II (STb)**
- **enteroaggregative heat-stable toxin type 1 (EAST1)**

- **shiga toxin type 2e (Stx2e)**

Question: *how prevalent were adhesins and toxins in ETEC causing PWD?*

Prevalence of virulence genes of *E. coli* strains and pathotypes associated with PWD





Zhang et al., 2007, Vet. Microbiol. 123:145-52

Virulence significance of enterotoxin in pig diarrhea

$3x10^9$ CFU bacteria inoculum;
K88ac receptor-positive or 987P-positive gnotobiotic piglets;
24 h post-inoculation;

isogenic strain	*diarrhea outcome*	*dehydration*	*colonization*
K88/LT	100%, severe	mild – severe	$1.21x10^{9**}$
K88/STb	67%, mild	no – light	$5.7x10^8$
K88 (-)	none	no	$5.9\ x10^8$
987P/STa	100%, severe	mild	$1.5\ x10^9$
987P(-)	none	no	$1.2x10^9$
K88/EAST1	none	no	$3x10^9$

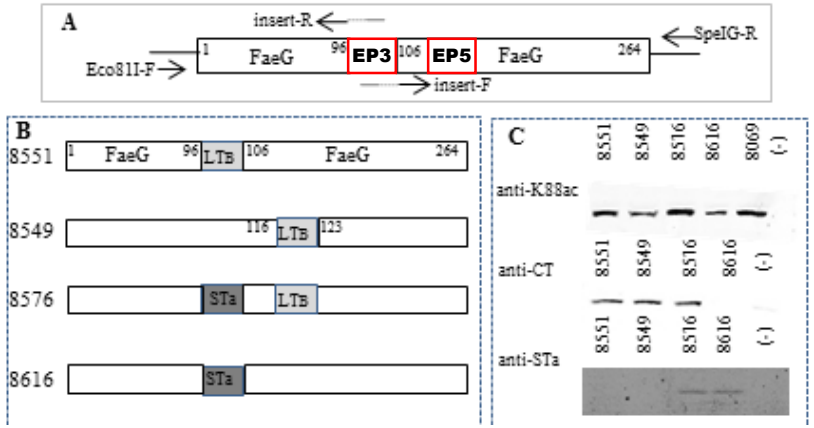
Stx2e/F18/ST ETEC associated with edema disease (ED).

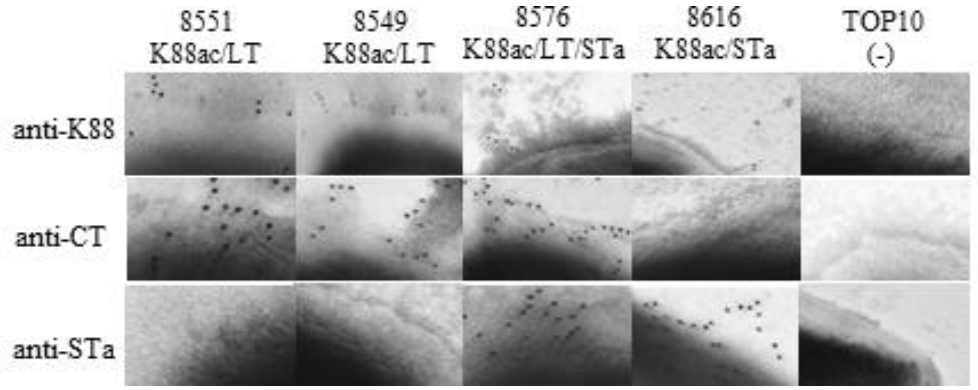
Target virulence factors: K88, F18, LT, STa, STb & Stx2e

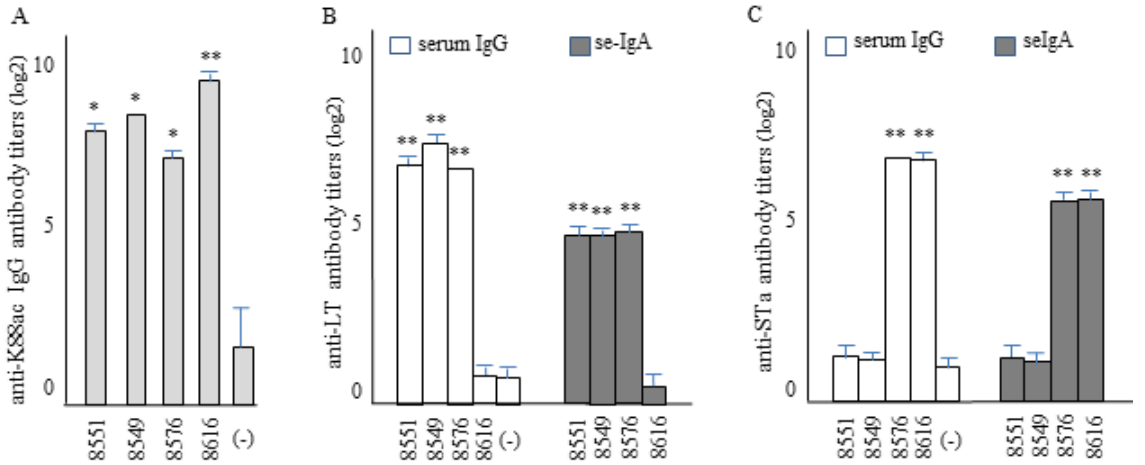
Vaccine strategy : 1) neutralizing epitopes, 2) MEFA backbone

Zhang et al., 2006. Infect. Immun. 74(6):3107-14;
Zhang et al., 2008. Appl. Environ. Microbiol. 74(18):5832-7;
Ruan et al., 2012, PLOS ONE 7(8):e43203.

K88ac fimbria platform to present toxins for antibodies against homologous adhesin and toxins

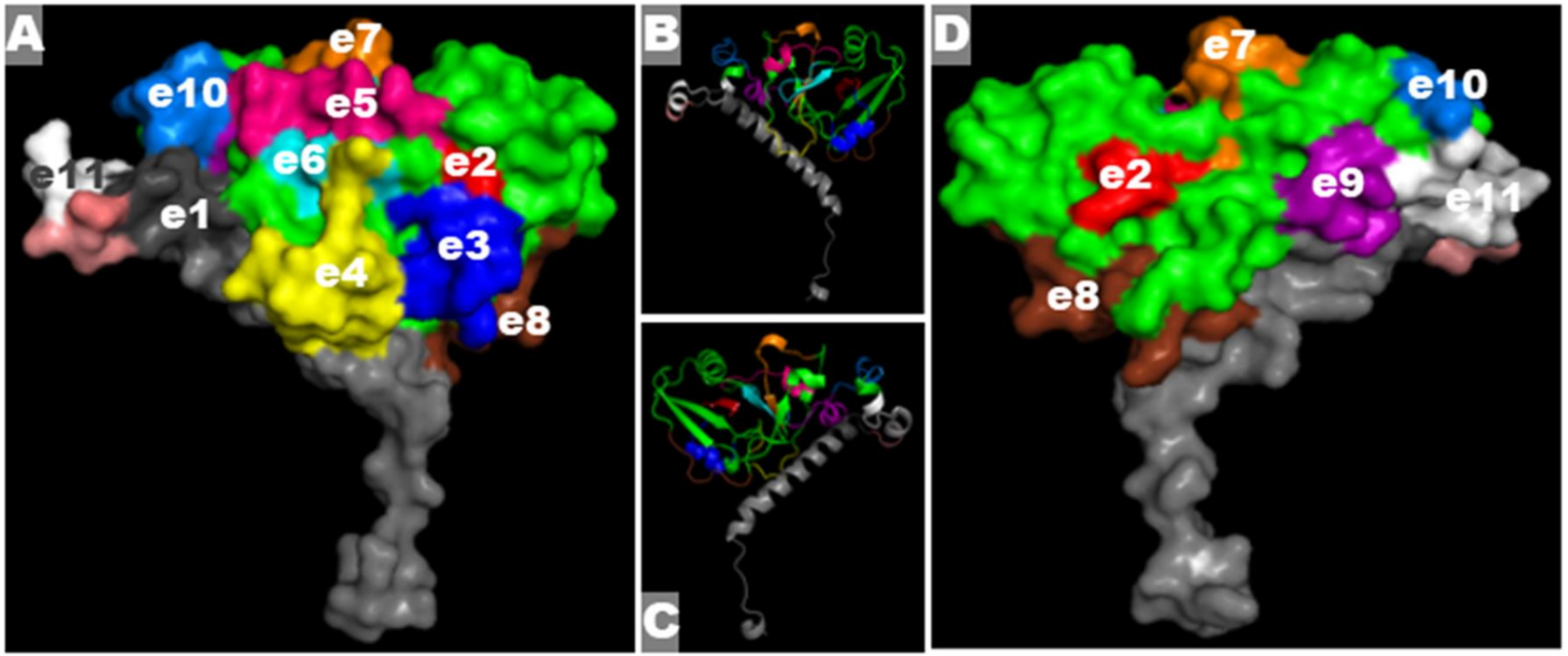






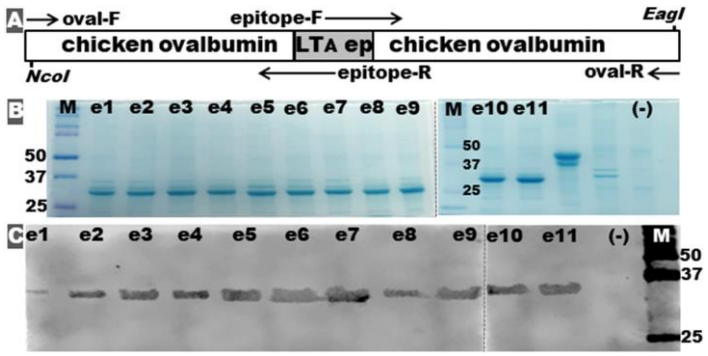
Zhang & Zhang, 2010; CVI 17(12):1859-67

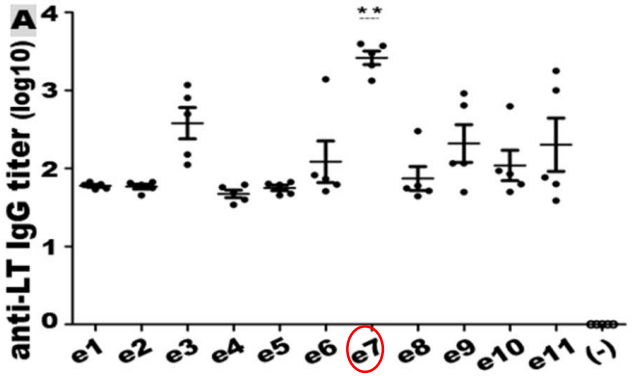
LT, an ideal MEFA backbone to present foreign epitopes & to mimic epitope native antigenicity

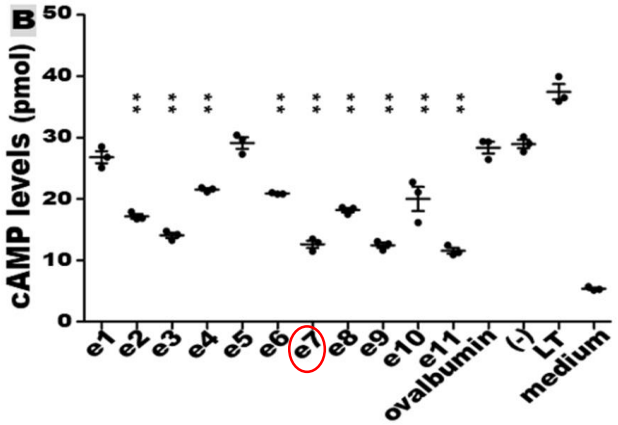


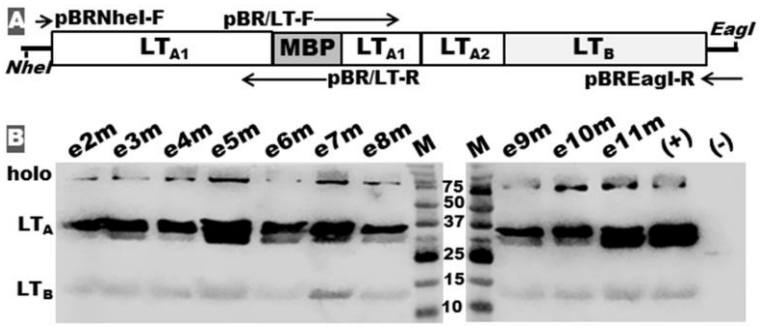
Huang et al., 2018, Appl. Environ. Microbiol. 84:e00849-18

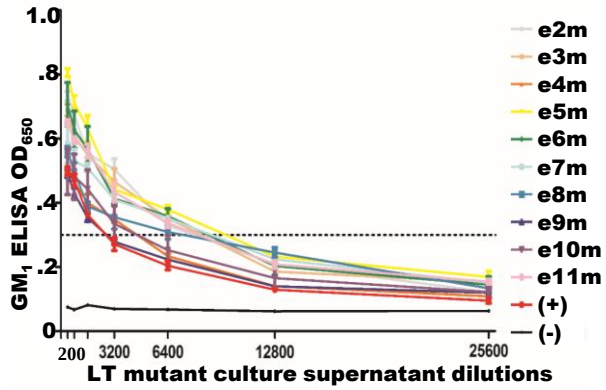
LT as MEFA backbone to present foreign epitopes and to mimic epitope native antigenicity

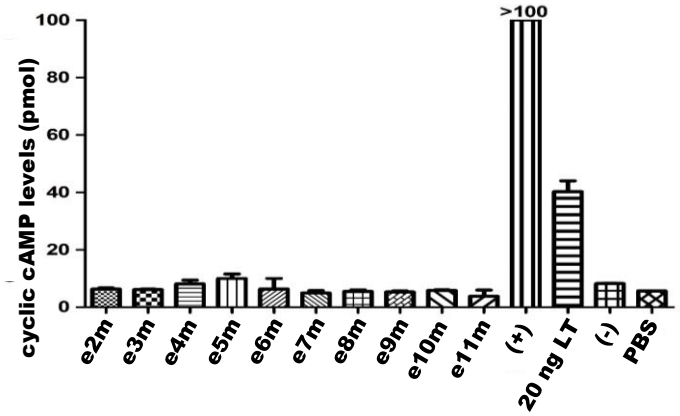






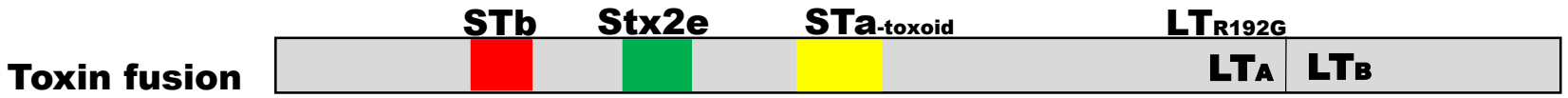


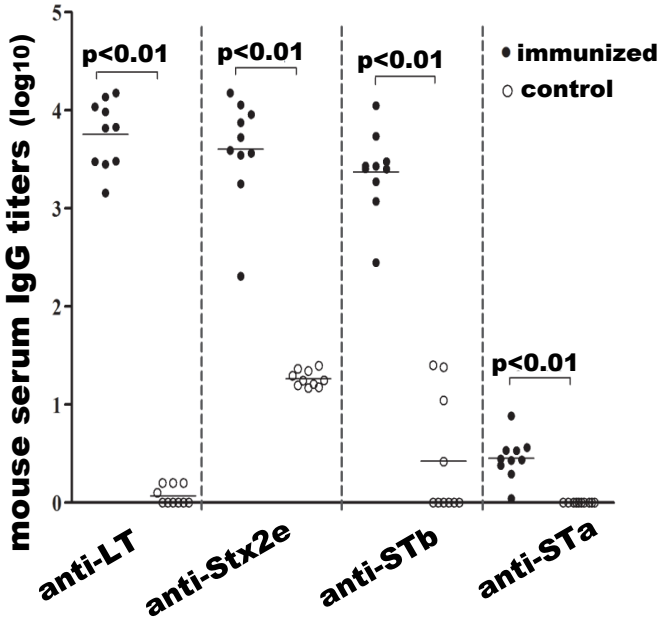


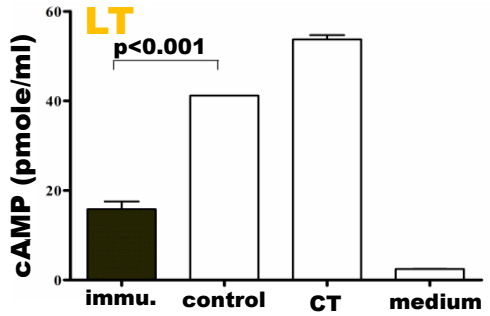


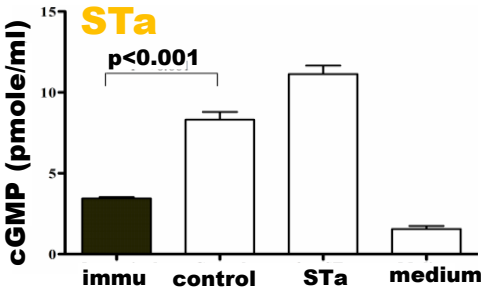
Huang et al., 2018, Appl. Environ. Microbiol. 84:e00849-18

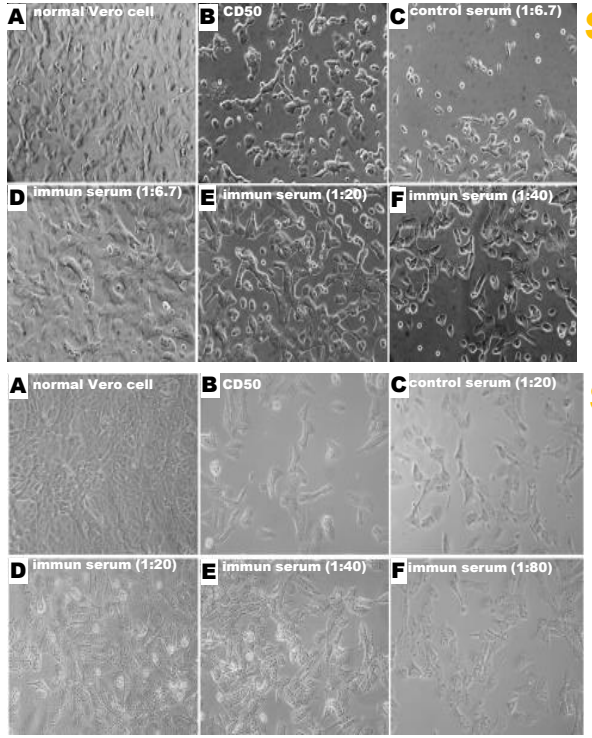
Neutralizing epitopes identified from ETEC toxins







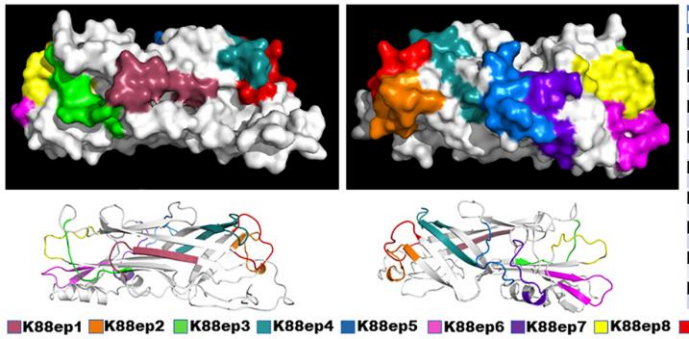




Rausch et al, 2017, Vet. Microbiol. 202:79-89

Neutralizing epitopes identified from K88ac fimbria

K88 FaeG



Ep #	AA sequence
K88ep1	^{23}MTGDFNGSVD^{32}
K88ep2	^{61}LNDLTNGGTK^{70}
K88ep3	^{84}GRTKEAFATP^{93}
K88ep4	^{115}ELRKPDGGTN^{124}
K88ep5	^{134}PMKNAGGTKVGAVKVN^{149}
K88ep6	^{157}LGRGGVTSADGEL^{168}
K88ep7	^{185}PRGSELSAGSA^{195}
K88ep8	^{233}RENMEYTDGT^{242}
K88ep9	^{262}FNQAVTTSTQ^{271}

Mouse serum anti-K88 IgG antibody titers (log_{10})

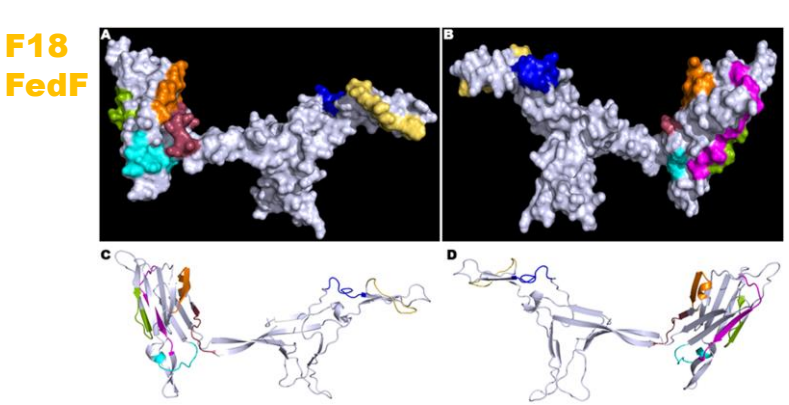
Mice immunized with	IgG titers	P values
ep1 fusion	3.61 ± 0.11	<.001
ep2 fusion	3.53 ± 0.25	<.001
ep3 fusion	3.45 ± 0.26	<.001
ep4 fusion	2.42 ± 0.40	<.001
ep5 fusion	3.89 ± 0.18	<.001
ep6 fusion	2.29 ± 0.30	<.001
ep7 fusion	2.08 ± 0.24	<.001
ep8 fusion	2.86 ± 0.08	<.001
ep9 fusion	2.85 ± 0.21	<.001
control	0 ± 0	

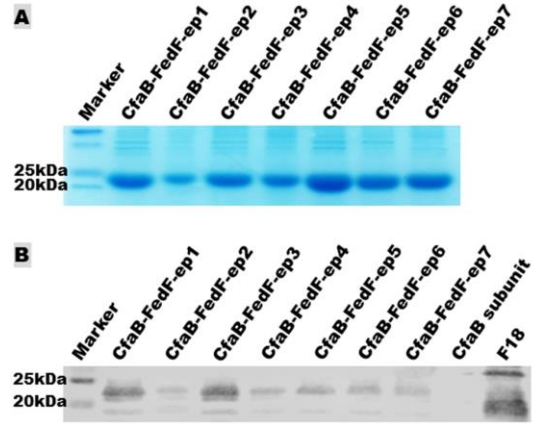
Mouse serum antibody inhibition against adherence of K88 fimbrial ETEC strain 3030-2 to porcine cell line IPEC-J2.

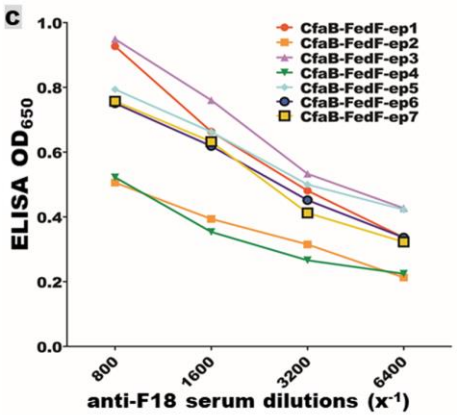
Mouse group	*Adherent bacteria (%)*	*P* value
control gourp	100 ± 24.2	
K88 fimbriae	9.8 ± 1.7	<0.001
ep1 fusion	41.5 ± 12	<0.001
ep2 fusion	44.3 ± 8.3	<0.001
ep3 fusion	37.2 ± 12	<0.001
ep4 fusion	28.5 ± 12.3	<0.001
ep5 fusion	22.5 ± 5.0	<0.001
ep6 fusion	87.5 ± 11.7	0.88
ep7 fusion	87.2 ± 15.2	0.86
ep8 fusion	66.7 ± 5.4	0.004
ep9 fusion	88.3 ± 23.5	0.92

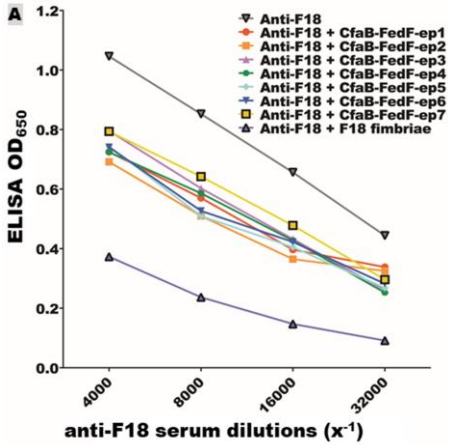
Lu et al, 2019, Appl. Environ. Microbiol.

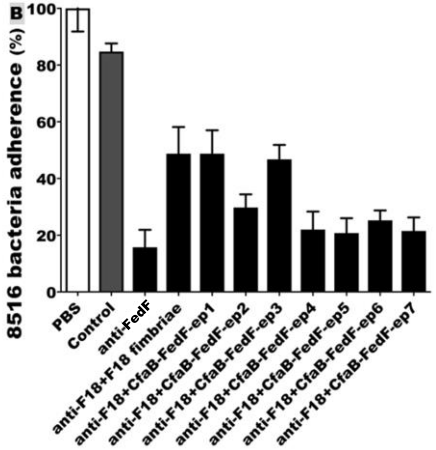
Neutralizing epitopes identified from F18ac fimbria

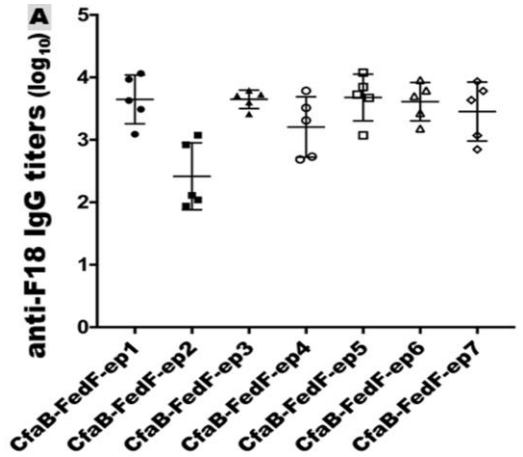


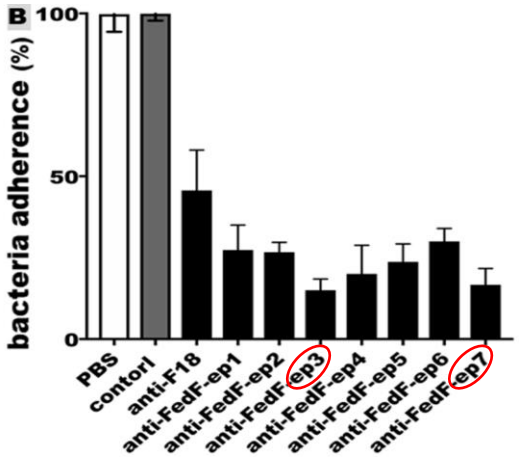












Lu et al, 2019, Vet. Microbiol. 230:171-177

MEFA vaccinology for a subunit vaccine against ETEC-associated children's & travelers' diarrhea

ETEC virulence factors in children's diarrhea and travelers' diarrhea

CFA adhesins

CFA/I

CFA/II (CS1, CS2, CS3)

CFA/IV (CS4, CS5, CS6)

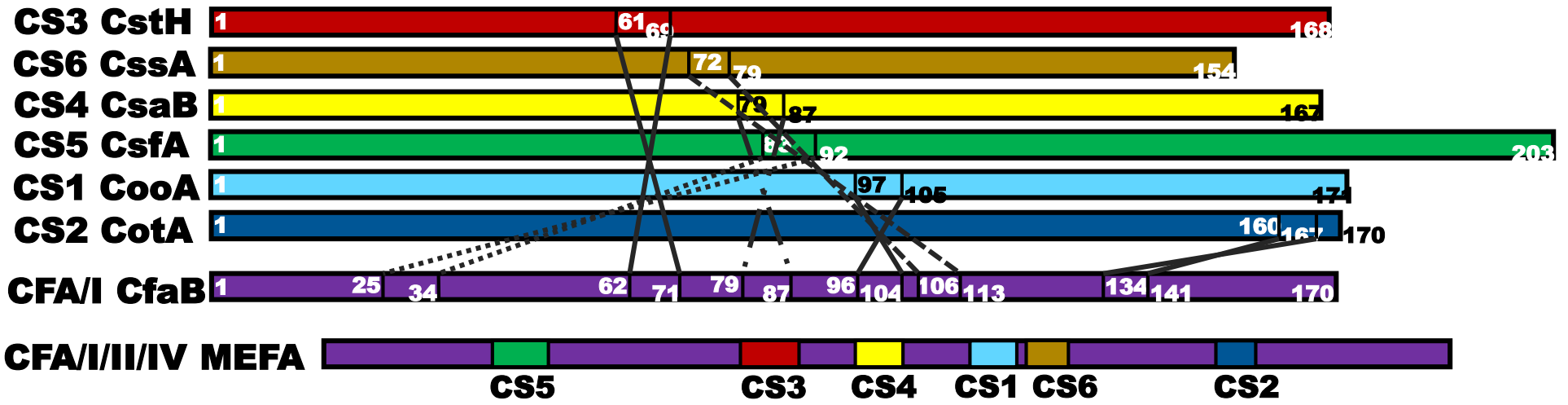
CS21, CS14, EtpA, EaeH …

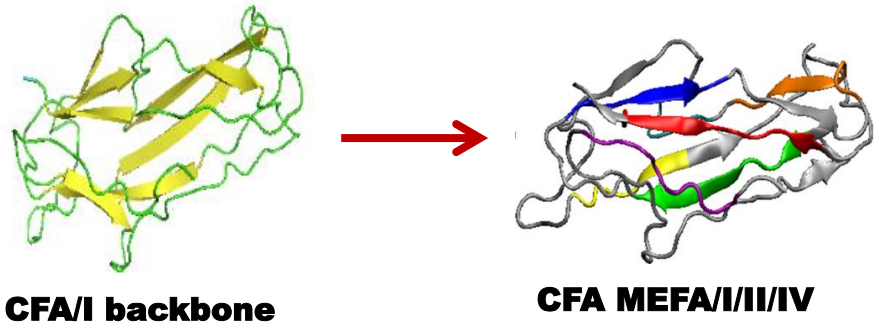
Toxins

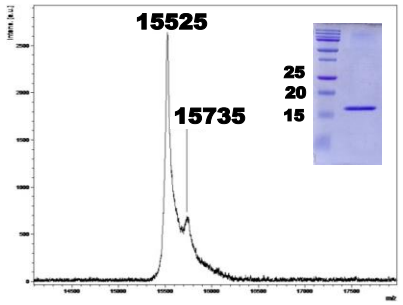
LT

STa

ETEC adhesin major subunit MEFA CFA/I/II/IV



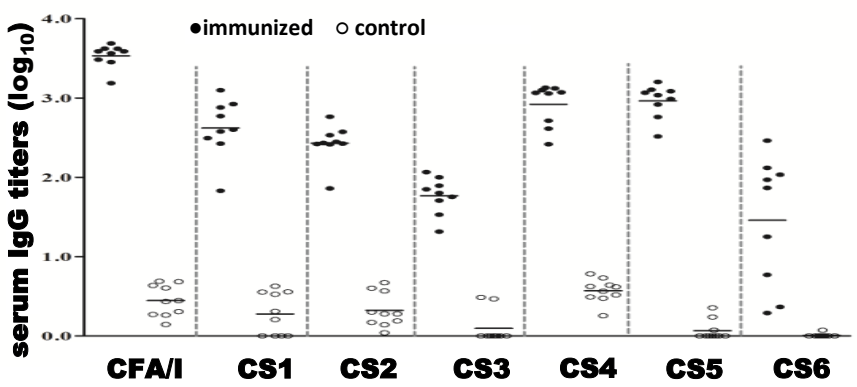


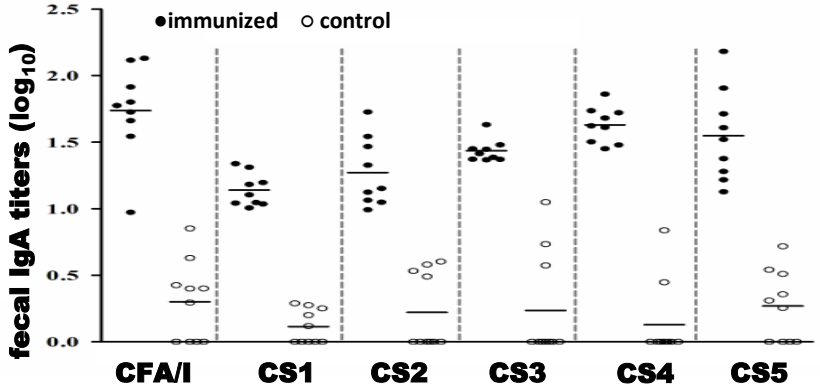


--- Ruan e al. 2014 CVI 21(2):243-249
--- Duan et al. 2017 JVV 8(4):1000367

ETEC adhesin major subunit MEFA CFA/I/II/IV

CFA/I/II/IV MEFA induces neutralizing antibodies against all 7 ETEC adhesins (CFA/I, CS1, CS2, CS3, CS4, CS5, and CS6)

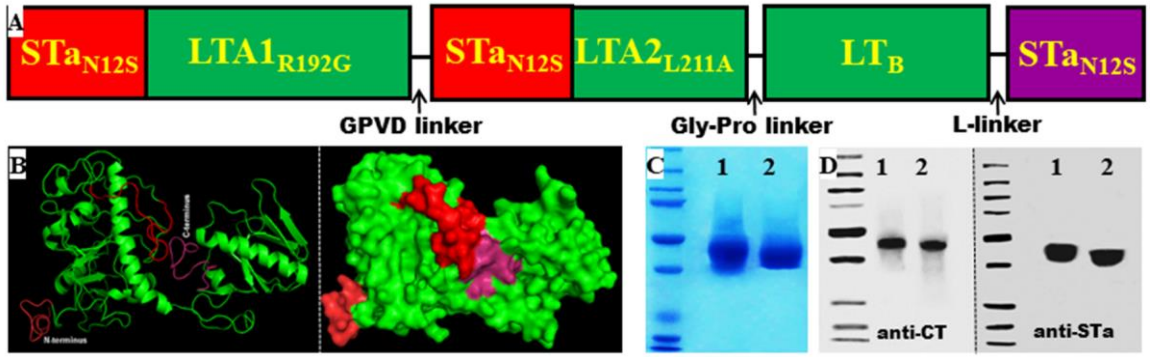


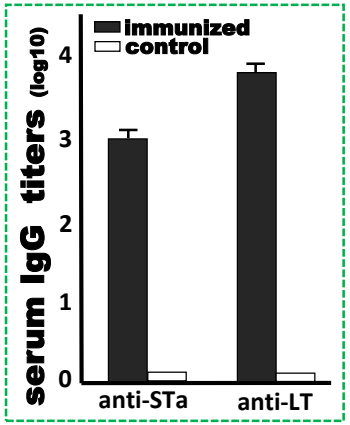


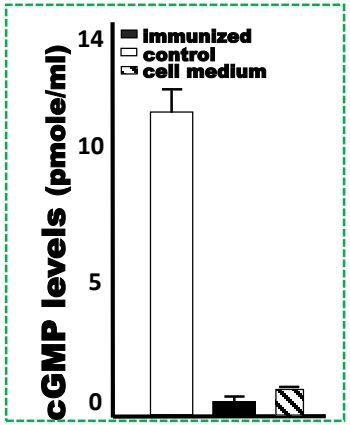
ETEC or *E. coli* strains	# of bacteria adherent to Caco-2 cells (x10^3)		
	immunized	control	p value
CFA/I ETEC (H10407)	24 ± 11	415 ± 144	<0.001
CS1 E. coli	8.6 ± 6.5	25 ± 14	<0.01
CS2 E. coli	14 ± 6.9	22 ± 7.1	0.03
CS3 ETEC (E116)	146 ± 20	295 ± 73	<0.001
CS4/6 ETEC (E106)	196 ± 50	398 ± 88	<0.001
CS5/6 ETEC (UM75688)	151 ± 41	352 ± 43	<0.01
CS6 (ETP98066)	96 ± 11.1	392 ± 8.7	<0.001

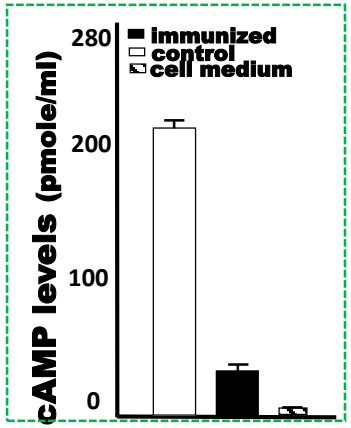
Ruan e al. 2014 CVI 21(2):243-249

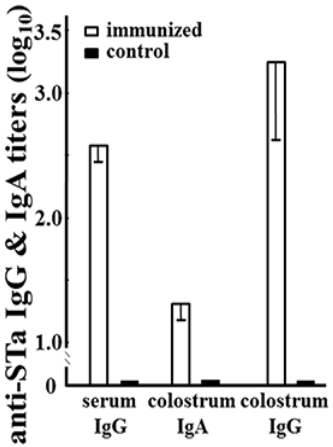
Toxoid fusion 3xSTa_{N12S}-$mnLT_{R192G/L211A}$ induced protective antibodies against LT and STa

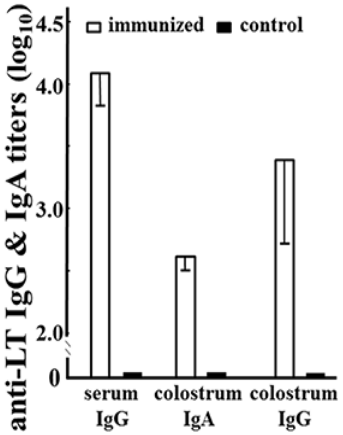


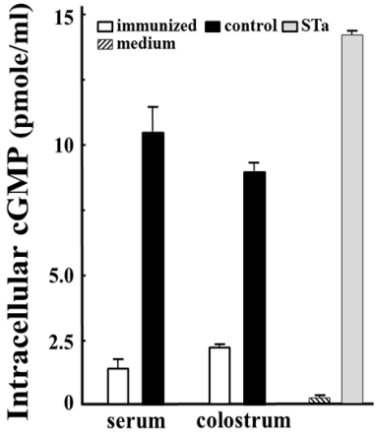










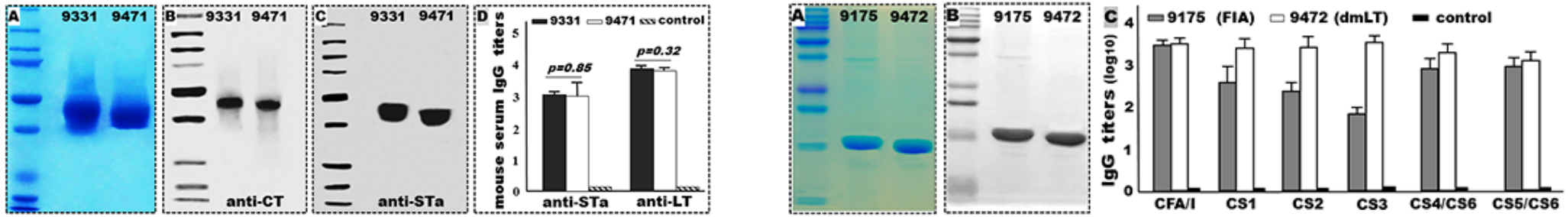


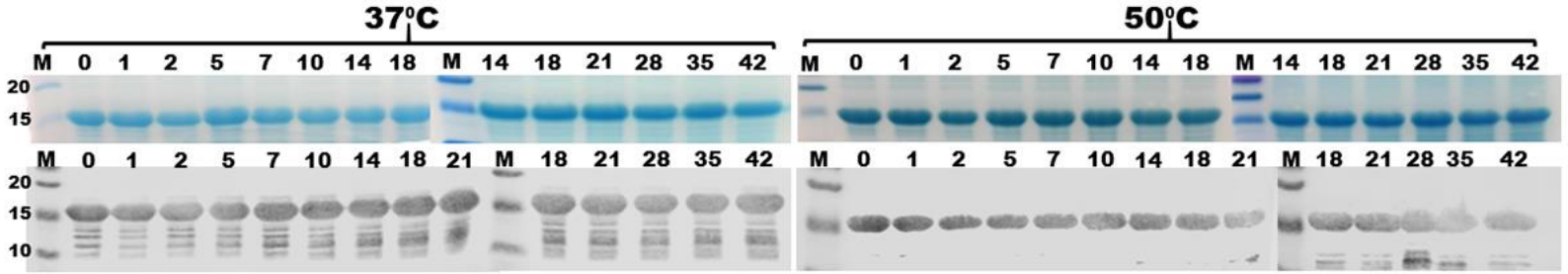
piglets	watery diarrhea	mild diarrhea	healthy	efficacy
STa+ ETEC challenge				
immunized	0	8	20	100% ~ watery diarrhea; 65% ~ any diarrhea.
control	26	0	6	
LT+ ETEC challenge				
immunized	1	0	7	
control	7	0	1	

--- Ruan et al. 2014 Infect Immun. 82:1823-32;
--- Nandre et al. 2017, Vaccine 35:552-6

MecVax, a multivalent enterotoxigenic *Escherichia coli* (ETEC) vaccine for children's and travelers' diarrhea

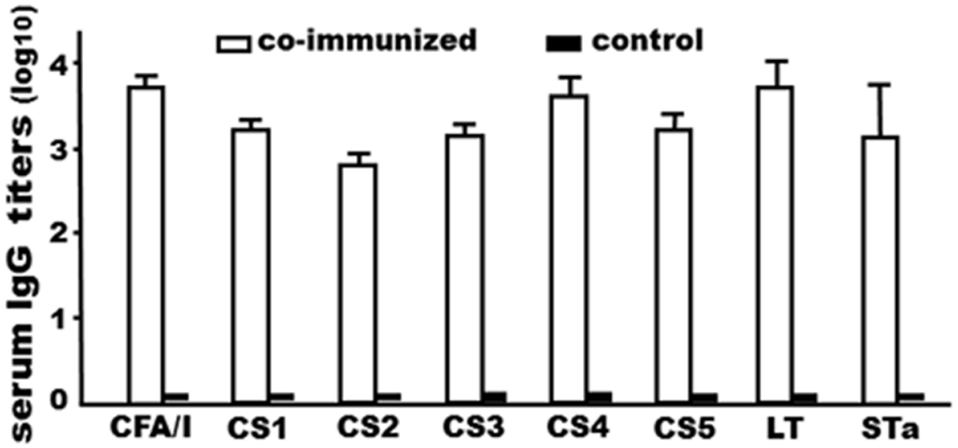
MecVax combined tag-less toxoid fusion 3xSTa$_{N12S}$-mnLT$_{R192G/L211A}$ and CFA/I/II/IV MEFA proteins





--- Duan et al., 2018, Front. Microbiol. 9:e1198

MecVax induced antibodies blocking adherence of the seven adhesins (CFA/I, CS1-CS6) neutralizing LT & STa



Antibody neutralizing CT and STa enterotoxicity

treatment	Cyclic AMP (pmol)		Cyclic GMP (pmol)	
STa or CT toxin	178.2 ± 4.8	P-value	35.3 ± 2.5	P-value
control serum	159.2 ± 0.7	0.11	34.5 ± 0.9	0.56
MecVax serum	19.8 ± 1.8	<0.01	1.6 ± 0.1	<0.01
culture medium	0.4 ± 0.1	<0.01	0.4 ± 0.0	<0.01

Antibody inhibiting CFA/I, CS1-CS6 adherence

Mouse groups	Number of bacteria (%) adhered to Caco-2 cells						
	H10407 (CFA/I)	THK38/pEU 405 (CS1)	DH5α/pEU 588 (CS2)	E116 (CS3)	E106 (CS4/CS6)	UM75699 (CS5/CS6)	ETP98066 (CS6)
Control	99.97±7	100±5	100±4.6	100±4.8	99.9±4.5	100±10.7	100±2
dmLT adjuvant	86.4 ± 7.8 p=0.01	82 ± 34.0 p<0.01	91 ± 5.0 p=0.19	91 ± 2.7 p=0.16	89 ± 14.8 p=0.06	88 ± 8.4 p=0.03	96.6 ± 8 p=0.97
MEFA w/ dmLT	62 ± 1.8 (p<0.01)	36 ± 3.3 (p<0.01)	57 ± 5.1 (p<0.01)	48 ± 2.7 (p<0.01)	53 ± 13.6 (p<0.01)	58 ± 4.1 (p<0.01)	25 ± 2.8 (p<0.01)
MecVax w/ dmLT	52 ± 15 (p<0.01)	32 ± 2.4 (p<0.01)	50 ± 4.8 (p<0.01)	55 ± 3.1 (p<0.01)	43.3 ±1.4 (p<0.01)	43 ± 3.4 (p<0.01)	30 ± 1.8 (p<0.01)

--- Duan et al., 2018, Front. Microbiol. 9:e1198

MecVax efficacy from rabbit-pig dual challenge model

	Pregnant gilts						Piglets born to	
	Serum IgG		Colostrum IgA		Colostrum IgG		Serum IgG	
	immunized	control	immunized	control	immunized	control	immunized	control
anti-STa	2.65 ± 0.25	0 ± 0	1.3 ± 0.26	0 ± 0	3.24± 0.63	0 ± 0	2.87 ± 0.04	0 ± 0
anti-LT	4.08 ± 0.24	0 ± 0	2.6 ± 0.22	0 ± 0	3.4 ± 0.68	0 ± 0	3.6 ± 0.29	0 ± 0

Clinic outcomes	Healthy	Mild diarrhea (pasty feces)	Watery diarrhea	Daily weight gain
immunized (n=16)	10	5	1	16%
Control (n=19)	2	0	17	7%

MecVax protects rabbits against ETEC colonization and pigs against ETEC diarrhea.

Summary

- MEFA platformed porcine ETEC antigen induces protective antibodies against K88, F18, LT, STa, STb, and Stx2e --- a broadly protective vaccine against porcine post-weaning diarrhea (PWD) and edema disease

- Adhesin and toxoid MEFAs induce protective antibodies against CFA/I, CS1-CS6, LT and STa, virulence determinants produced by ETEC strains that cause a majority of cases of children's diarrhea and travelers' diarrhea

- MEFA, the epitope- and structure-based vaccinology can be applied for development of broadly protective multivalent vaccines, against heterogeneous strains or different diseases

Acknowledgements

Zhang Laboratory

Postdoc: Mei Liu, Xiaosai Ruan, Rahul Nandre, Qiangde Duan, Hyesuk Seo, Nan Xiao.
Graduate students: Chengxian Zhang, Dana Rausch, Katie M Wollenberg, Jiachen Huang, Ti Lu, Carolina Garcia.

Drs. David Francis (SDSU), Rodney Moxley (UNL), Tom Casey (NADC), Dieter Schifferi (UP), Richard Isaacson (UMN)

David A Sack (Johns Hopkins)

STa Toxoid Vaccine Consortium Group: JP Nataro (UVA), E Barry (Maryland), JD Clements (Tulane), W Zhang (KSU), H Sommerfelt (Bergen, Norway)

Dr. Donald C Robertson (KSU)
Dr. James Fleckenstein (Washington Univ.)
Dr. June Scott (Emory University)
Dr. Ann-Mari Svennerholm (Univ. of Gothenburg, Sweden)

Funding Sources:

NIH: AI068766; AI076871; AI083897; AI109209-02; AI101302-01; AI127050; AI121067-01; HHSN2722012000051
PATH/BMGF: STa toxoid vaccine consortium phase I, II, III (2009-2018)
USDA: NIFA (2017-67015-26632, NRICGP SD00224-G), AES (SD00H391-11; SD00H288-08IHG)
South Dakota State University, Kansas State University, University of Illinois at Urbana-Champaign