

# **Clinical trials of an oral inactivated ETEC vaccine (ETVAX) in children in developing countries and in travelers**

Ann-Mari Svennerholm

Department of Microbiology and Immunology  
The Sahlgrenska Academy  
University of Gothenburg (UG)



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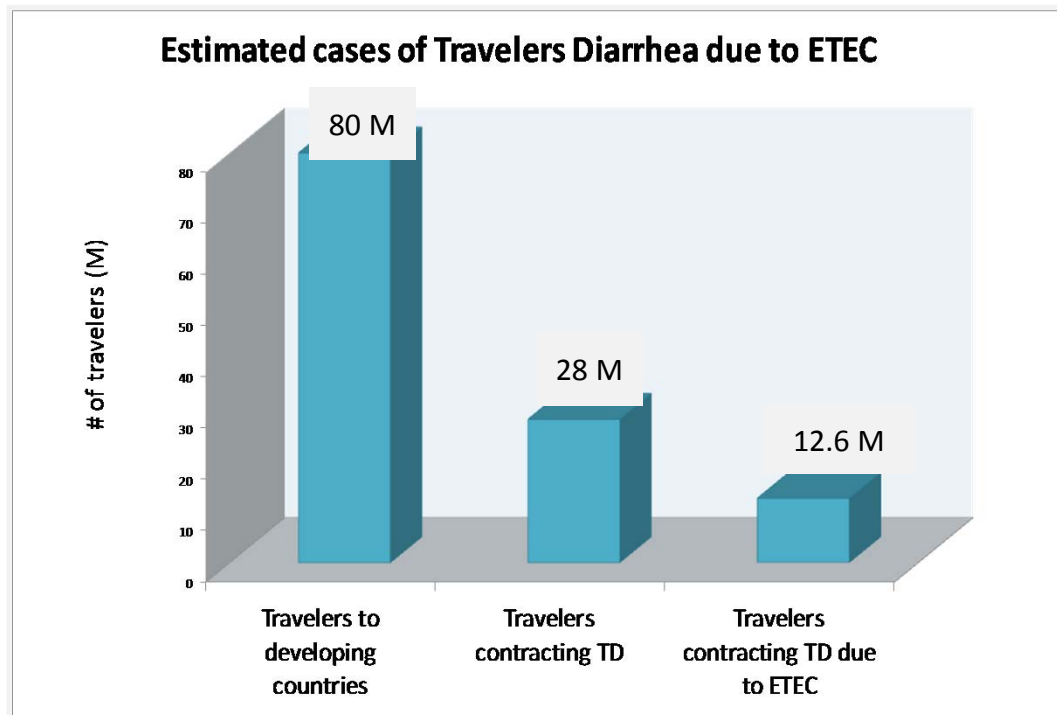
# Enterotoxin-producing *E. coli* (ETEC) – important global diarrheal pathogen in humans

ETEC is one of the most common causes of diarrhea in children  $\leq 5$  years in developing countries

- ETEC causes several hundred million cases and >50,000 diarrheal deaths annually
- Repeated ETEC diarrheas may cause undernutrition, retarded growth and decreased cognitive functions



ETEC is also the most common cause of diarrhea in travellers



20 - 50 % of all diarrheas in travellers are caused by ETEC

## Why is no human ETEC vaccine available yet?

Large heterogeneity of diarrheagenic ETEC and lack of robust immunological markers of protection makes vaccine development complicated



### Enterotoxins:

LT (~40%), ST (STh and STp; ~30%), LT+ST (~30%)

### Colonization factors (CFs):

> 25 CFs; CFA/I and **CS1-CS7**, **CS14** and **CS17** on 50-80% of all ETEC strains (Gaastra & Svennerholm 1996)

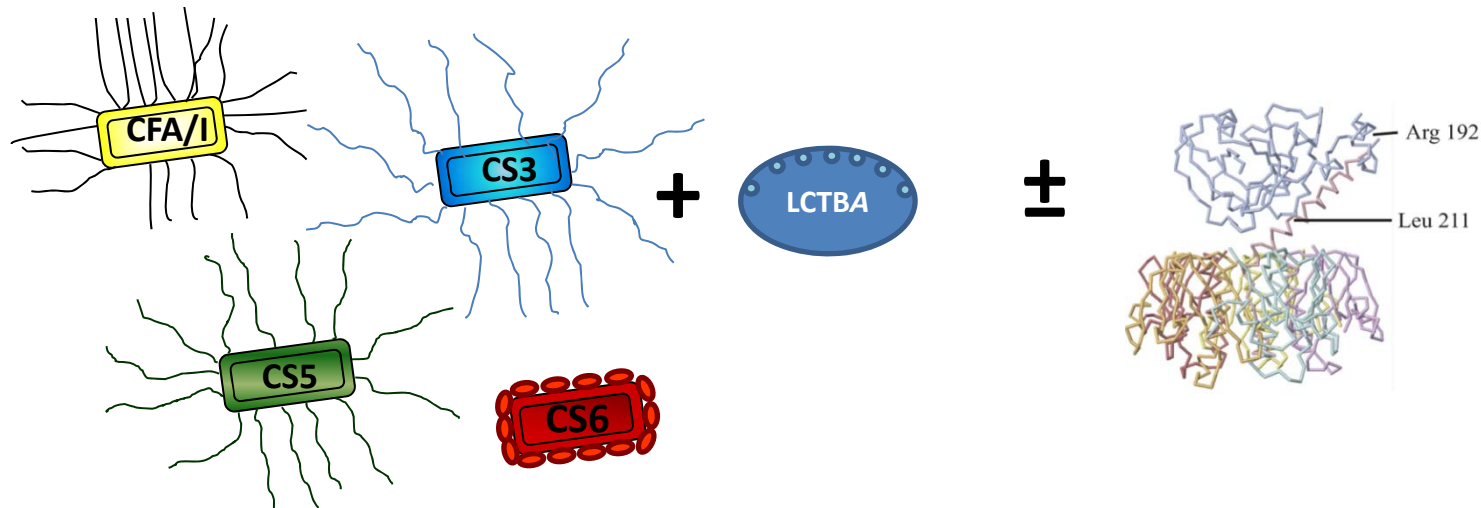
**O-antigens:** >70 in ETEC

**Putative protective surface antigens:** e.g. EtpA, EatA, flagellin, TibA (Fleckenstein et al)

# The ETEC vaccine, ETVAX, developed at UG in collaboration with Scandinavian Biopharma, Stockholm

Multivalent ETEC vaccine (ETVAX)

dmLT adjuvant



**$10^{11}$  recombinant bacteria  
overexpressing CFs  
(Tobias & Svennerholm)**

**+1mg toxoid  
(Lebens , Holmgren et al)**

**10  $\mu$ g dmLT  
( J Clements et al)**

# Evaluation of ETVAX in Phase I double-blind placebo- controlled studies in adult Swedes

Four groups with altogether 129 subjects receiving:

- A. Placebo (bicarbonat buffer only); n=34
- B. ETVAX alone; n=35
- C. ETVAX + 10 µg of dmLT; n=30
- D. ETVAX + 25 µg of dmLT; n= 30

Boosting groups A-C with single dose ETVAX 1-2 years later

## Is ETVAX safe ?

- No significant differences in side-effects between vaccinees and placebos

## Can ETVAX induce?:

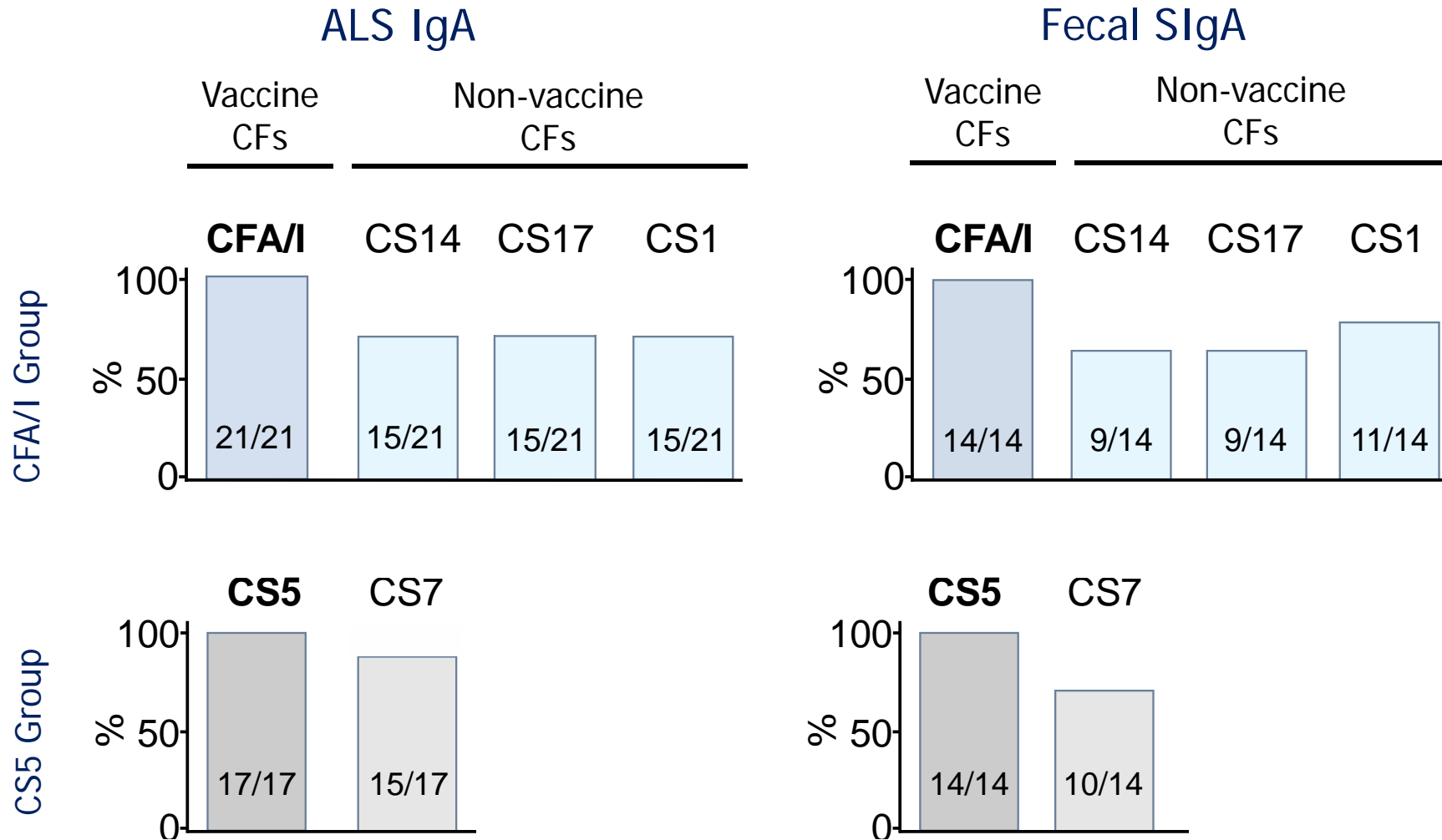
- **Mucosal immune responses:** SIgA antibody responses fecal extracts and ...intestine - derived circulating antibody secreting cell (ASC/ALS IgA) responses
- **Serum antibody responses** against the primary vaccine antigens
- **Cross-reactive immune responses** against other CFs than those in the vaccine
- **Immunological memory**

## Mucosal ALS IgA and/or fecal SIgA immune responses against ETVAX antigens in adult Swedes

Frequencies of responders to two oral doses of ETVAX against the five primary vaccine antigens (CFA/I, CS3, CS5, CS6 and LTB)

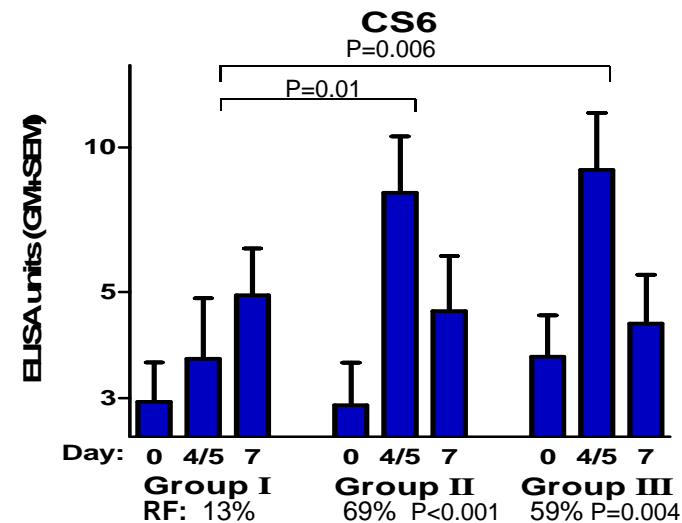
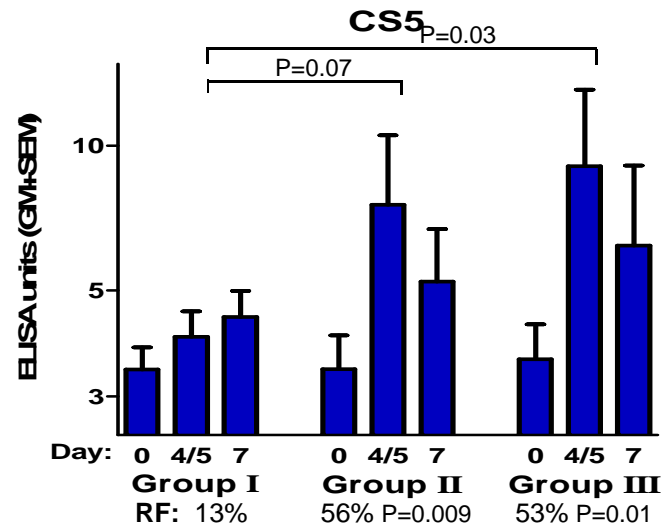
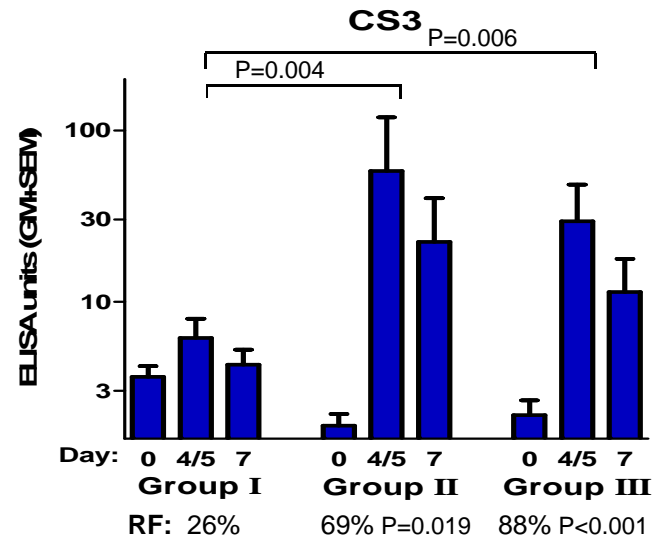
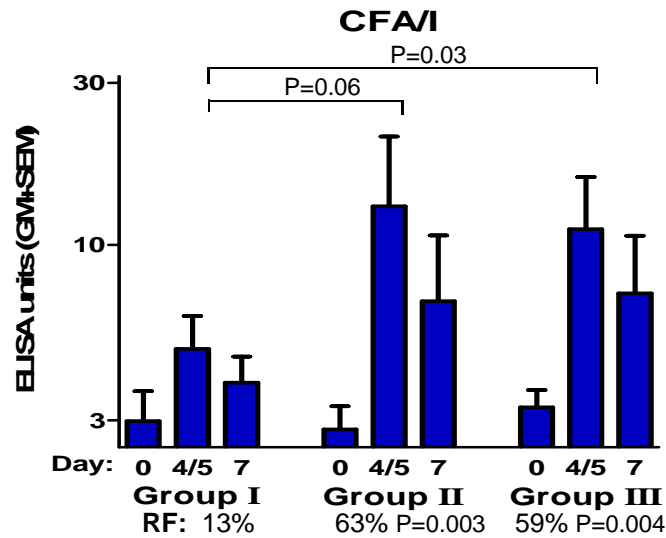
Subjects responding to	Placebo	ETVAX alone	ETVAX + 10 µg dmLT	ETVAX + 25 µg dmLT	All Vaccinees n=95
≥ 4 antigens	0%	91%	87%	85%	87%
5 antigens	0%	74%	83%	75%	77%

# Induction of cross-reactive mucosal immune responses against CFs not included in the vaccine in adult Swedes



## Immunological memory responses:

Higher and earlier appearing ALS responses were induced by a single dose of ETVAX in previously (1-2 years earlier) vaccinated (Gr II and III) compared to in naive (Gr I) Swedish adults





# Phase I/II study of ETVAX in descending age groups in Bangladesh

## Main Objective :

**Safety:** To identify the highest safe and immunogenic dose of ETVAX +/- dmLT adjuvant

## Secondary objective:

**Immunogenicity:** To determine immune responses against the five primary ETVAX antigens (CFA/I, CS3, CS5, CS6 and LTB)



## Mucosal immune responses

- IgA antibodies in ALS specimens
- SIgA /total SIgA antibodies in fecal extracts

## Systemic immune responses

- IgA and IgG antibodies in plasma

Can dmLT adjuvant enhance immune responses?

# Phase 1/II trial of ETVAX in Bangladesh (495 subjects )

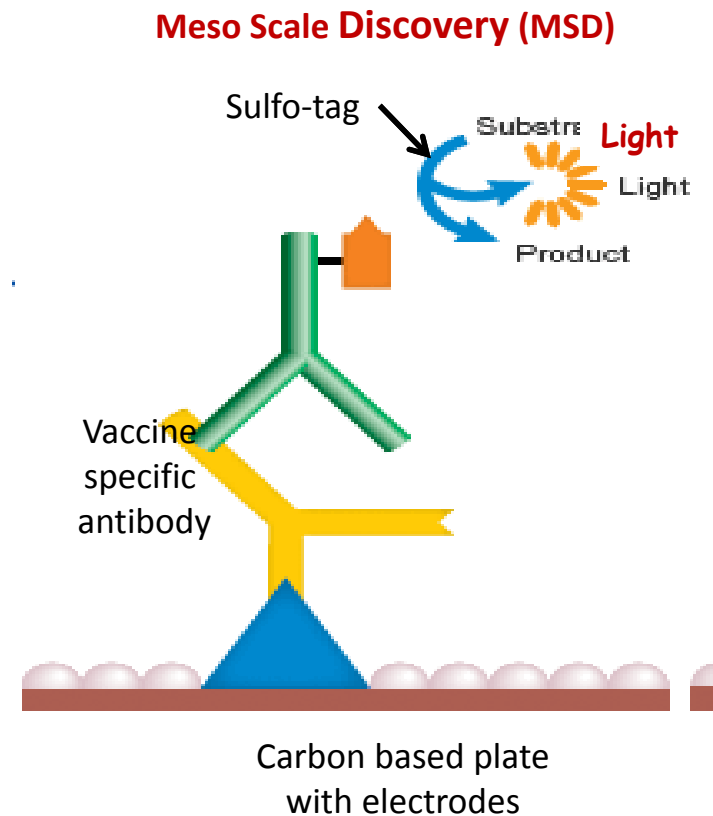
<b>PART A: 18-45 years, inclusive</b>	<b>Cohort</b>	<b>ETVAX</b>	<b>dmLT</b>	<b>N</b>
	A1	Full dose	-----	15
		Full dose	10 ug	15
		Placebo		15
Total			45	

<b>PART B: 24-59 months, inclusive</b>	<b>Cohort</b>	<b>ETVAX</b>	<b>dmLT</b>	<b>N</b>
	B1	¼ dose	-----	15
		Placebo	-----	10
	B2	½ dose	-----	15
		Placebo	-----	10
	B3	Full dose	-----	15
		Placebo	-----	10
	B4	½ dose	2.5 µg	15
		Placebo	-----	10
	B5	½ dose	5 µg	15
		Placebo	-----	10
	B6	½ dose	10 µg	15
		Placebo	-----	10
	Total			150

<b>PART C: 12-23 months, inclusive</b>	<b>Cohort</b>	<b>ETVAX</b>	<b>dmLT</b>	<b>N</b>
	C1	¼ dose	-----	15
		Placebo	-----	10
	C2	½ dose	-----	15
		Placebo	-----	10
	C3	½ dose	2.5 µg	15
		Placebo	-----	10
	C4	½ dose	5 µg	15
		Placebo	-----	10
	Total			100

<b>PART D: 6- 11 months, inclusive</b>	<b>Cohort</b>	<b>ETVAX</b>	<b>dmLT</b>	<b>N</b>
	D1	1/8 dose	-----	15
		Placebo	-----	10
	D2	¼ dose	-----	15
		Placebo	-----	10
	D3	½ dose	-----	15
		Placebo	-----	10
	D4	¼ dose	2.5 µg	15
		Placebo	-----	10
	D5	¼ dose	5 µg	15
Placebo		-----	10	
Total			200	

# Introduction of a new approach to assess ALS responses by an electrochemiluminescence assay

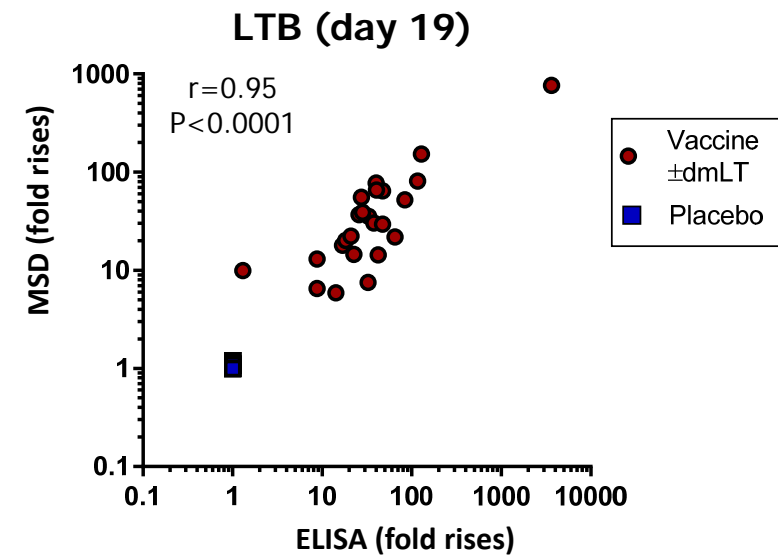
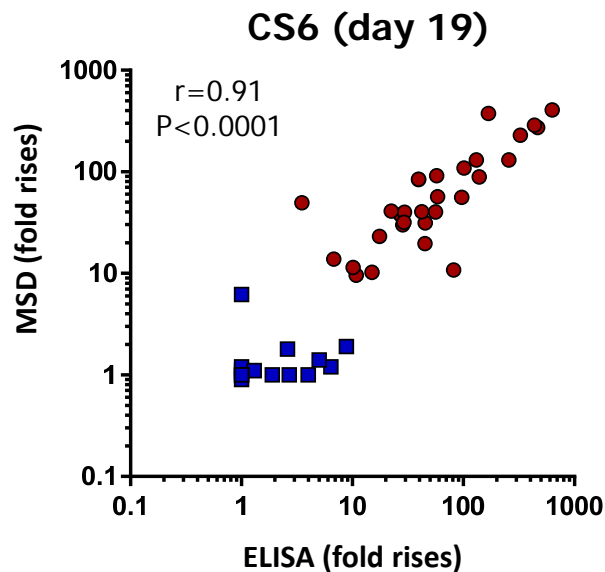
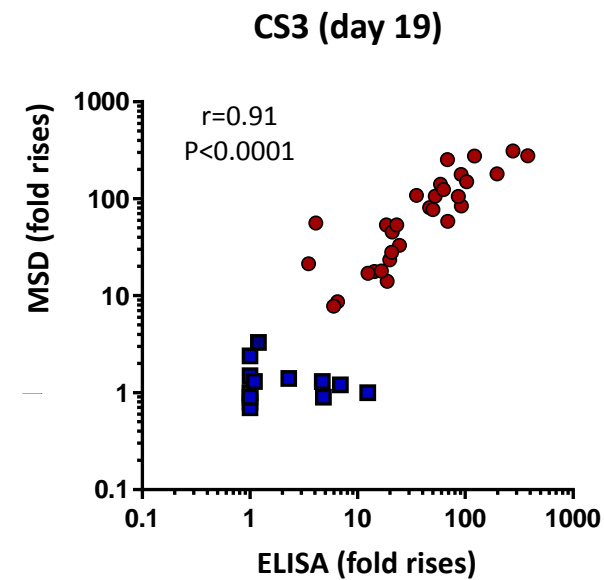
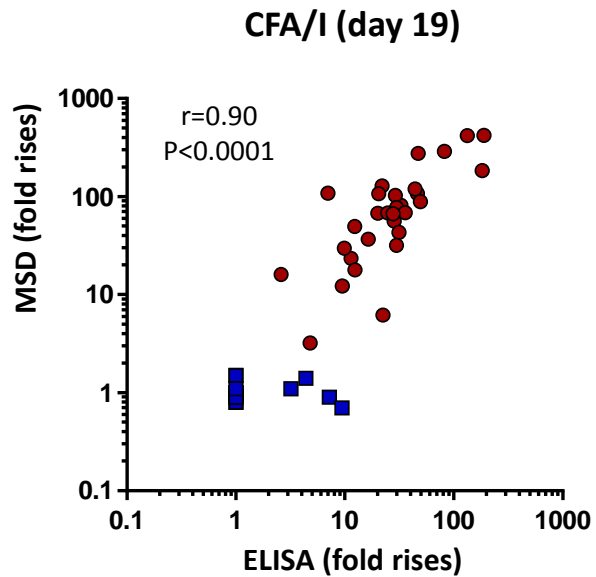


Main benefits of ECL assays compared to ELISA:

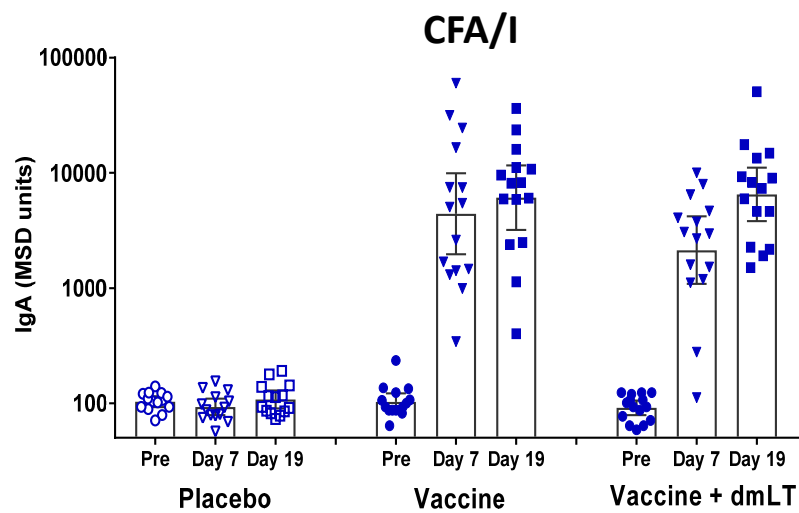
- High sensitivity – small sample volumes required (5  $\mu$ l/analysis), e.g for use in young children
- Wide dynamic range – no sample titration required

# Excellent correlation between ALS IgA responses by ELISA and ECL MSD assay

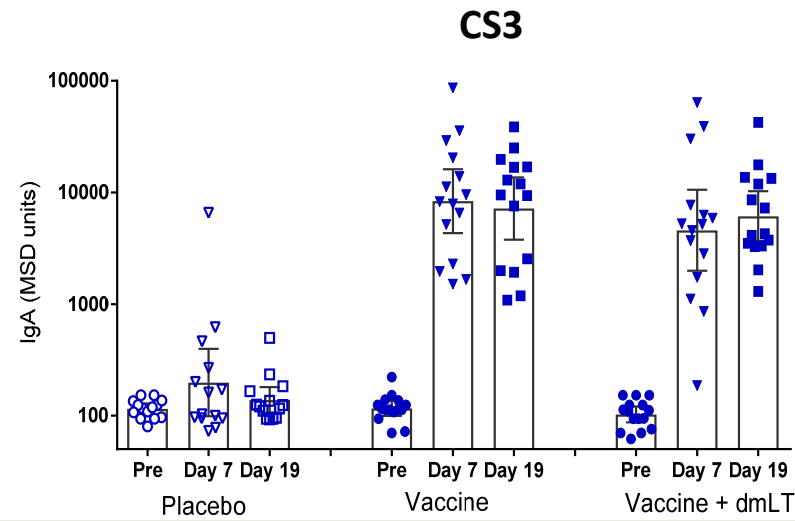
(Immune responses to CFs and LTB in adult Bangladeshis given ETVAX d ± dmLT or placebo)



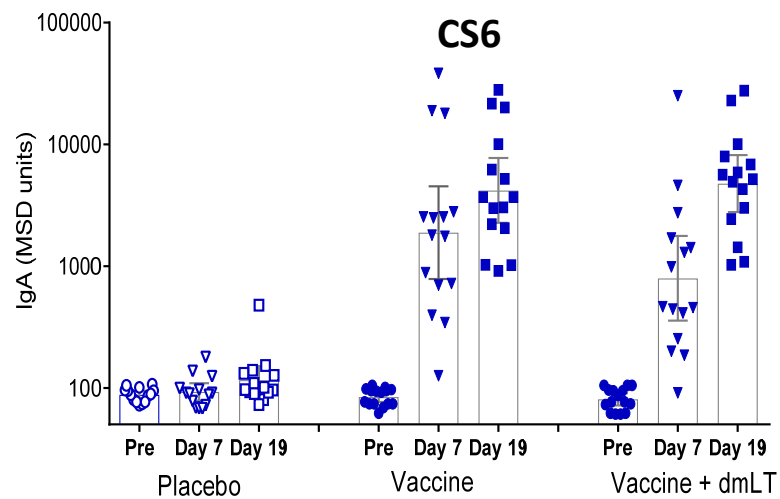
## Strong and specific ALS IgA responses against CFs and LTB in adult Bangladeshis given two doses of ETVAX ± dmLT or placebo (determined by the ECL MSD assay)



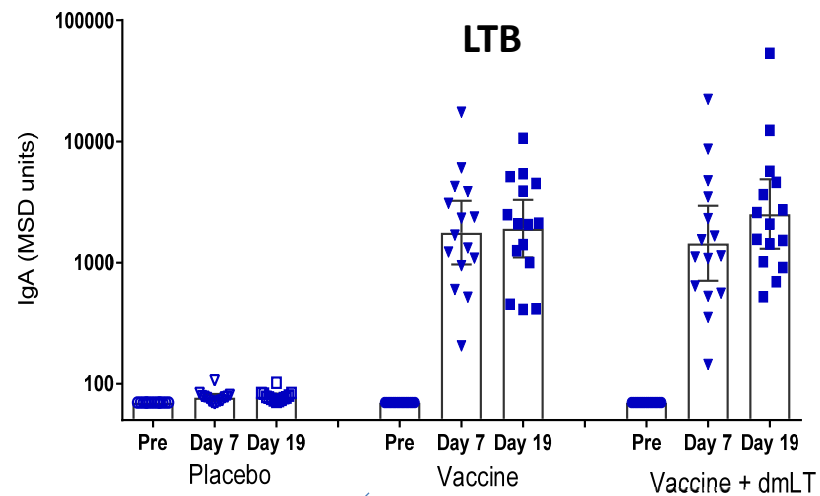
Responder freq:    0%                    100%                    100%



Responder freq:    7%                    100%                    100%



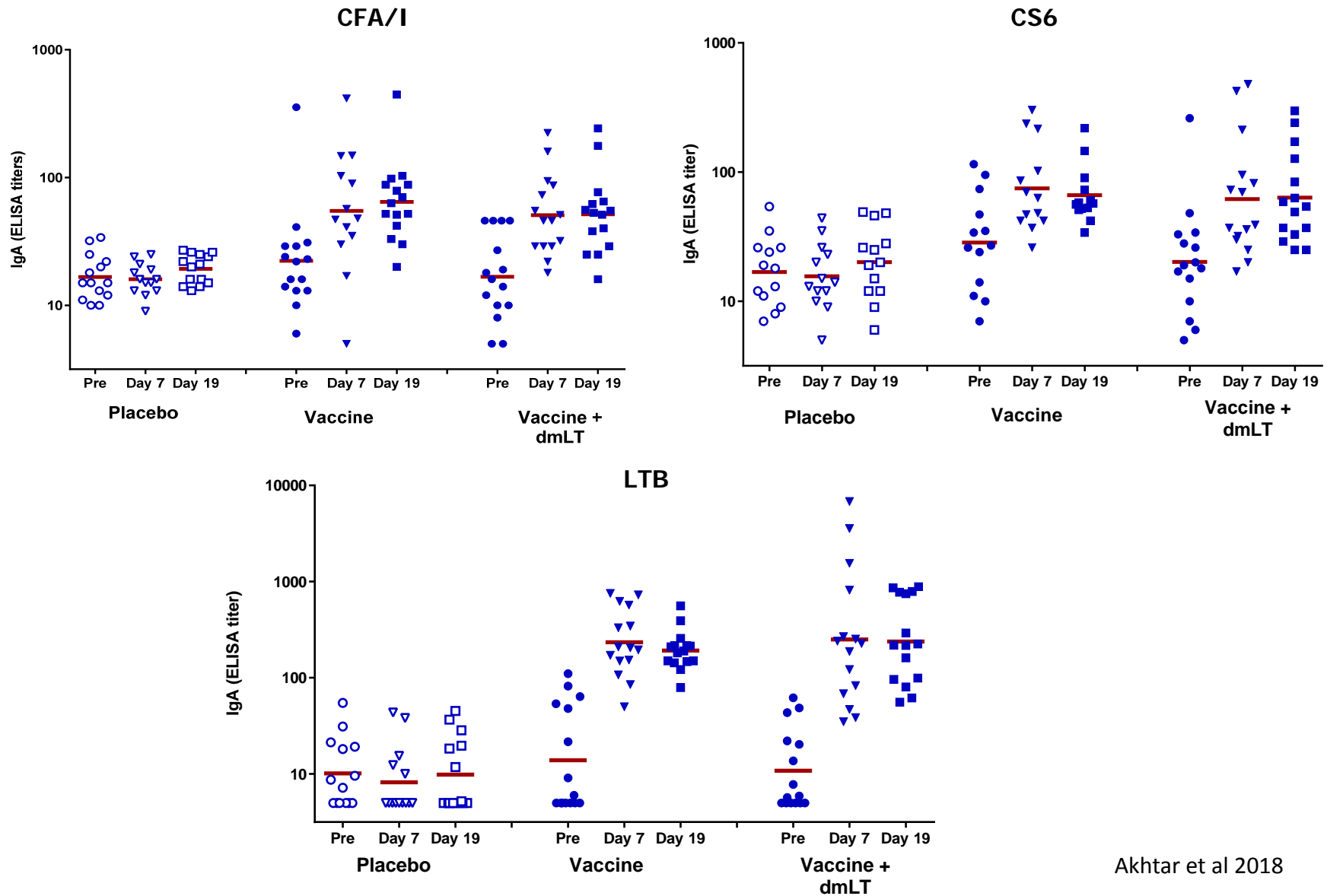
Responder freq:    7%                    100%                    100%



Responder freq:    0%                    100%                    100%

Also strong responses to CS5

# Frequent IgA responses in plasma against CFs and LTB in adult Bangladeshis: given two doses of ETVAX +/- dmLT or placebo day 0 and 14



## A phase 2b trial of ETVAX in Finnish travelers to Benin

(PI Anu Kantele, University of Helsinki)



A double – blind, placebo-controlled study in 800 volunteers to evaluate:

- Safety
- Immunogenicity (serum antibody responses )
- Protection against mild-moderate ETEC diarrhea with ETEC as only pathogen

Volunteers were given 2 doses of ETVAX or placebo (buffer only) 2 weeks apart in Finland 1-2 weeks before departure

Surveillance for diarrhea was conducted during stay in Benin for 12 days + one week after return to Finland

# Thank you!



GU, Institute of  
Biomedicine,  
Sahlgrenska  
Academy

**ETEC group icddr;**  
F Qadri et al



Stiftelsen för Strategisk forskning



L Bourgeois, R Walker,  
N Bauer et al



B Sjöstrand &  
N Carlin



Jan Holmgren

**ETEC group GU**

