

Human cellular and humoral immune responses against
colonization factors and mucinase YghJ in
experimental infection with
an epidemiologically relevant STh-only ETEC strain

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ST-ETEC important in childhood diarrhea

Levine *et al.* (1993)

- Santiago, Chile
- LT/ST- and ST-only ETEC strains
highest relative risk of isolation in
children with diarrhea

Steinsland *et al.* (2002)

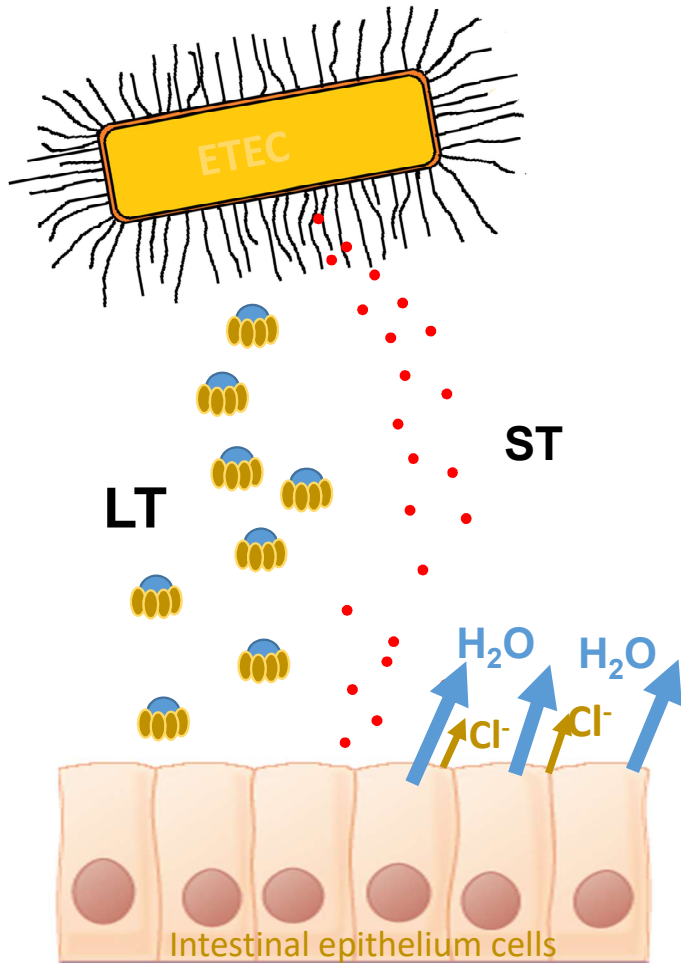
- Guinea-Bissau
- Infections with STh- and STh-LT-ETEC
associated with diarrhea
- Pathogenicity: STh-containing ETEC
>>> STp-containing ETEC

Porat *et al.* (1998)

- Southern Israel
- ST-containing ETEC: Significantly
more common in cases than in
controls
- Only STh associated with diarrhea

Kotloff *et al.* (2013)

- Global Enteric Multicenter Study
- ST-ETEC (with/without LT)
significantly associated with MSD
- ST-ETEC associated with increased
risk of case death in infants



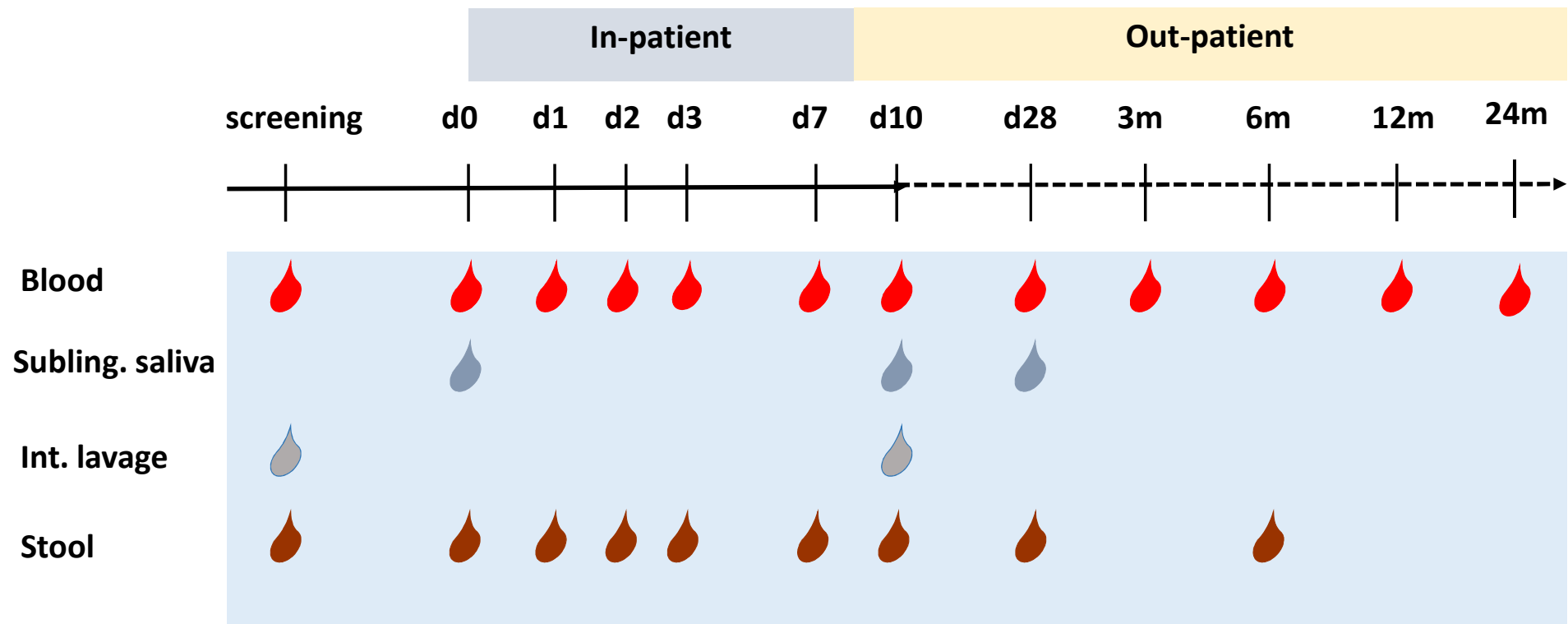
Aim

- ST is a relevant target for vaccine development
- Controlled human infection model can speed up testing of an ST-based vaccine candidate
- ST-only strain needed to avoid secretory diarrhea contributions from LT
- Secondary objective: Describe cellular and humoral immune response in natural ETEC infection

Controlled experimental infection study

- **Healthy adult volunteers - consent**
- Screening
 - No travels to ETEC endemic areas (≤ 12 months)
 - No immunosuppressive drugs
 - Normal immunoglobulins
 - Normal baseline blood values
 - No enteropathogens in stool
 - Intestinal lavage
- In-patient period 5-9 days after drinking ETEC inoculation dose
- Treatment Ciprofloxacin at d5 or before
- Discharge after three negative stool samples
- Mostly medical students
- Mostly females (87%)
- Mean age 23 (range 20-29)

Specimen collection



ETEC strain selection process

- Evaluation of phylogenetic data (*Hans Steinsland*)
- Strains from epidemiologically relevant ETEC families
- Representative strain within its family
- Isolated from a child with diarrhea
- Susceptible to at least one oral antibiotic

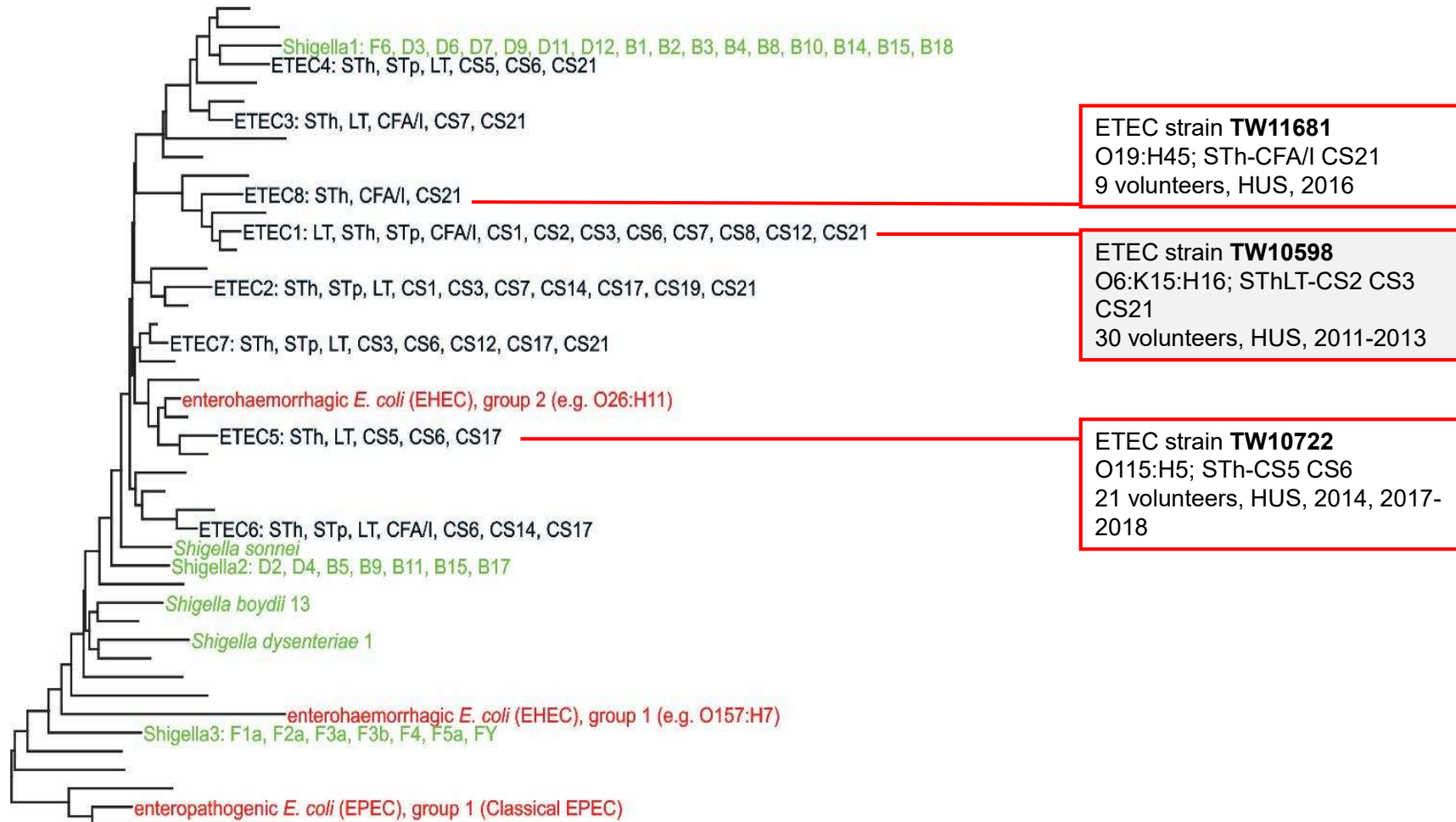
TW10722

- Wild-type STh-only ETEC strain
- Serotype O115:H5
- CS5, CS6, EtpA-, EatA+

TW11681

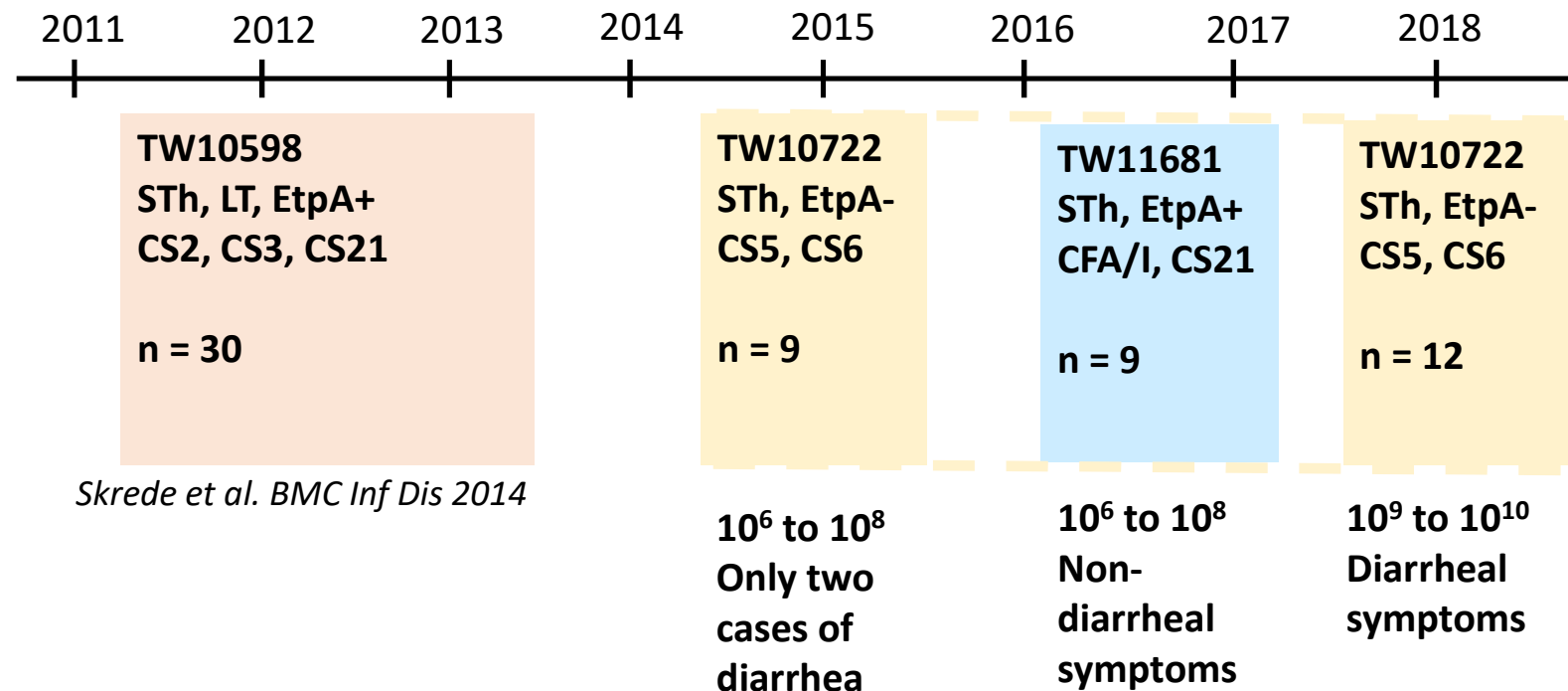
- Wild-type STh-only ETEC strain
- Serotype O19:H45
- CFA/I, CS21, EtpA+, EatA+

Strains used in Bergen

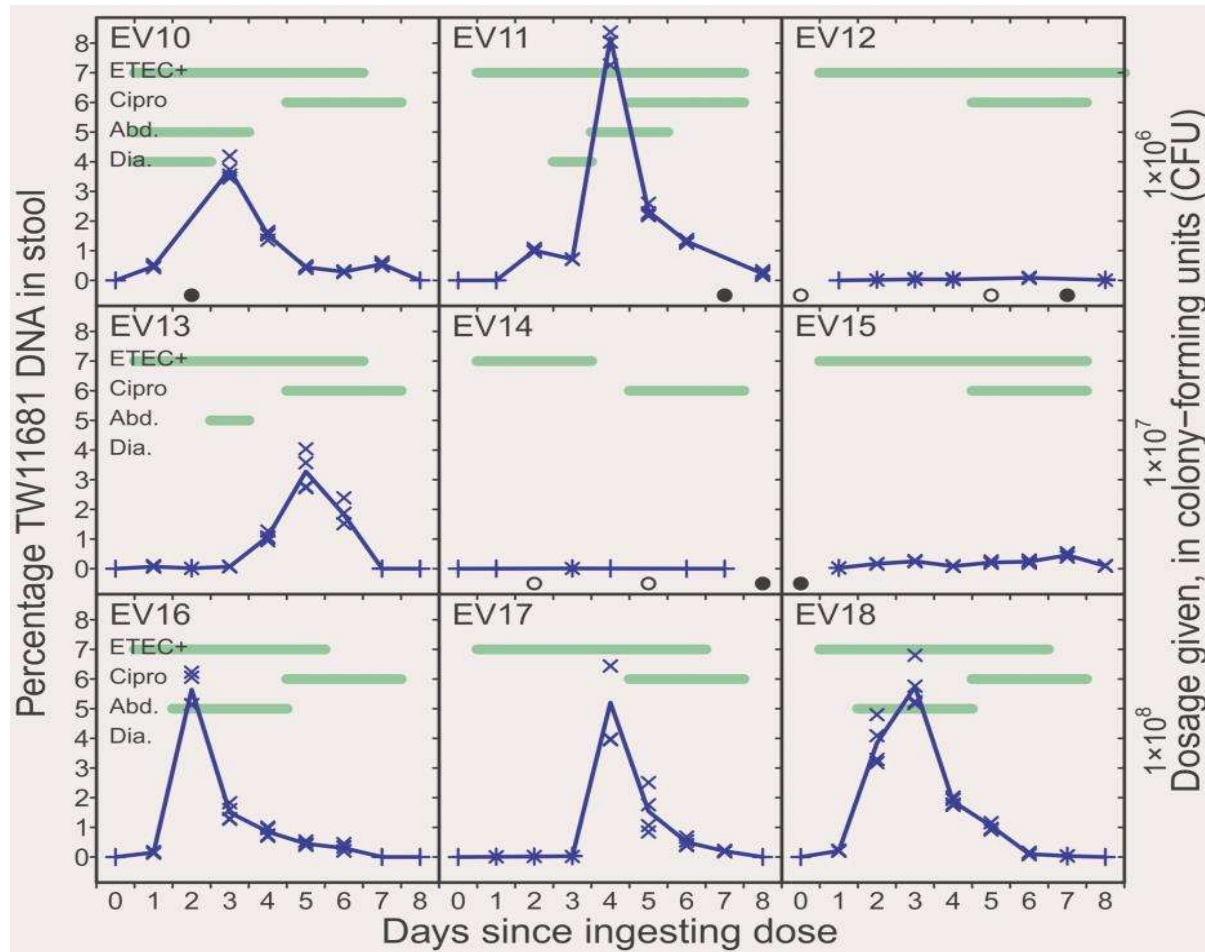


ETEC challenges in Bergen, Norway

Haukeland University Hospital & Univerisity of Bergen



qPCR of stool from TW11681 infected volunteers



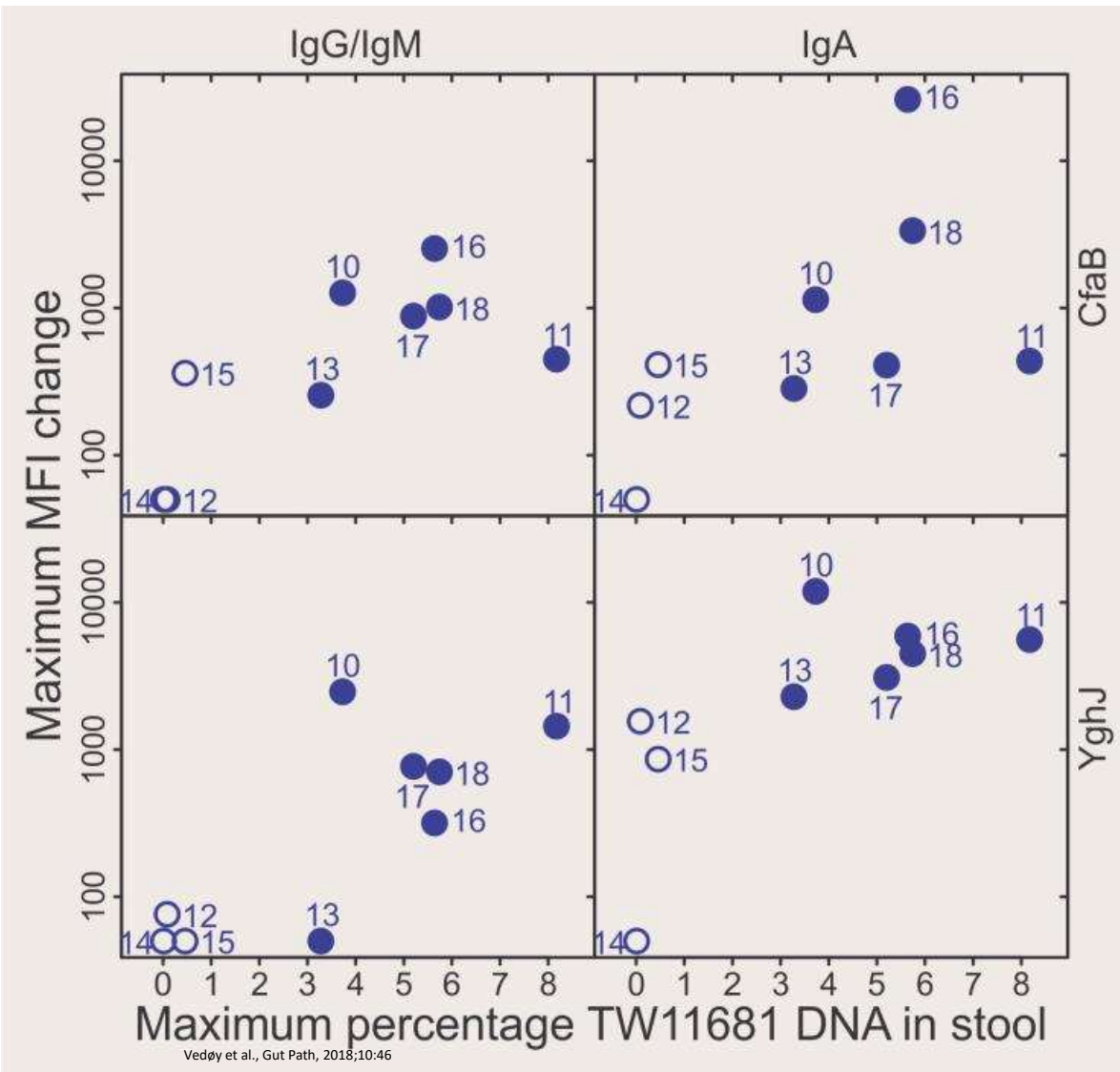
O - No stool passed; ● - Specimen not analyzed; "ETEC+" - Microbiologically positive for ETEC; "Cipro" - Antibiotic treatment; "Abd." - Abdominal pains or cramps; "Dia." - Diarrhea (mild)

- Six volunteers had TW11681 proliferation peaks.
- Three volunteers had no discernable peaks.
- All volunteers who had abdominal pains/cramps had peaks (5 of 6).

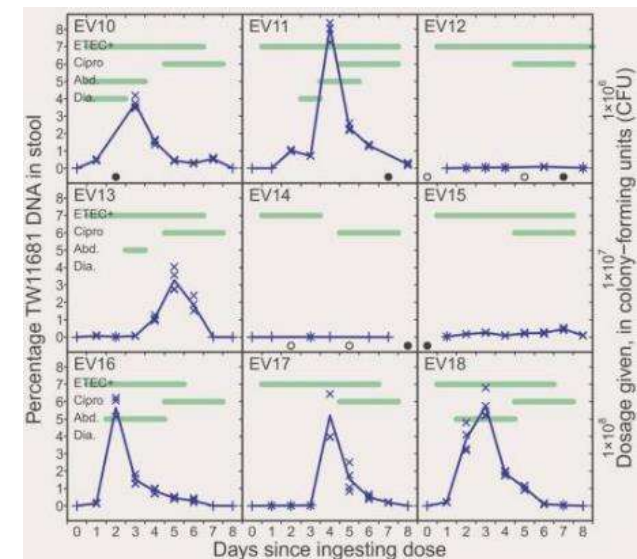
Changes in antibody levels

Maximum change in serum anti-CfaB and YghJ IgG/IgM and IgA levels - day 0 vs. day 10+28.

Closed (●) and open (○) circles = 6 and 3 volunteers who did and did not have peaks, respectively.



Vedø et al., Gut Path, 2018;10:46



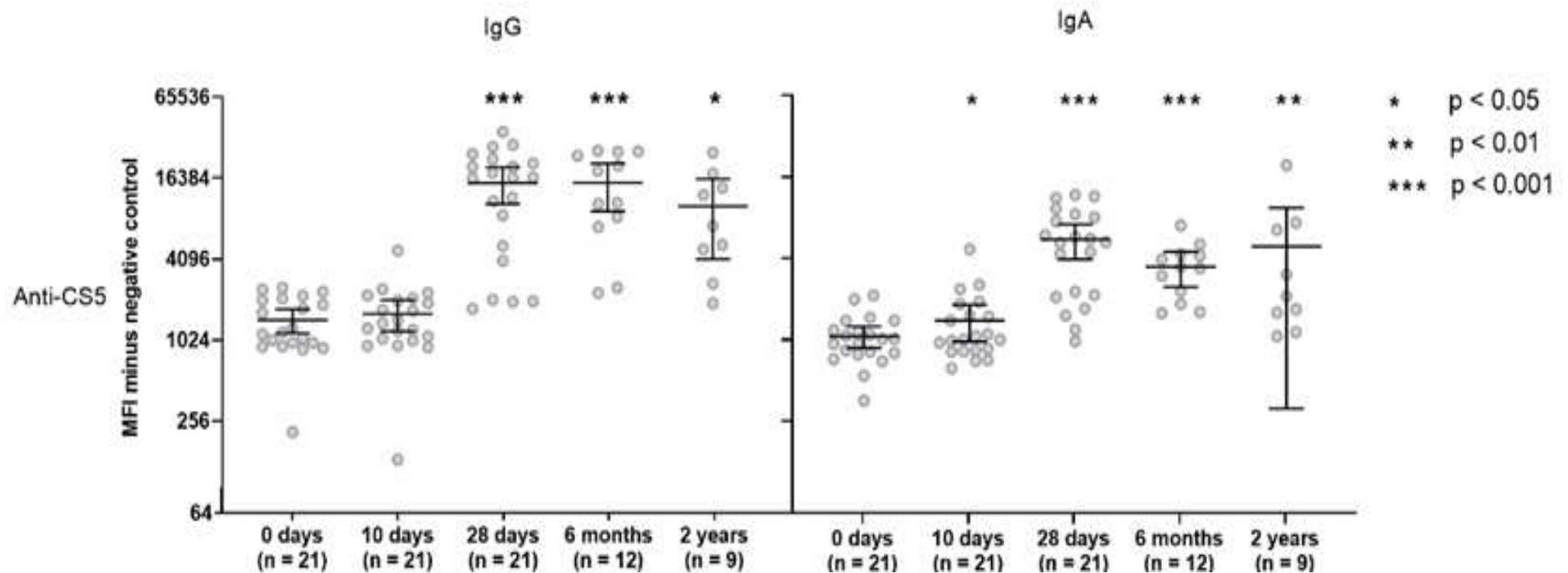
Symptoms and signs TW10722

Dose (CFU)	No of volunteers	No with diarrhea	Attack risk
1×10^6	3	0	0%
1×10^7	3	1	33%
1×10^8	3	1	33%
1×10^9	3	1	33%
1×10^{10}	9	7	78%

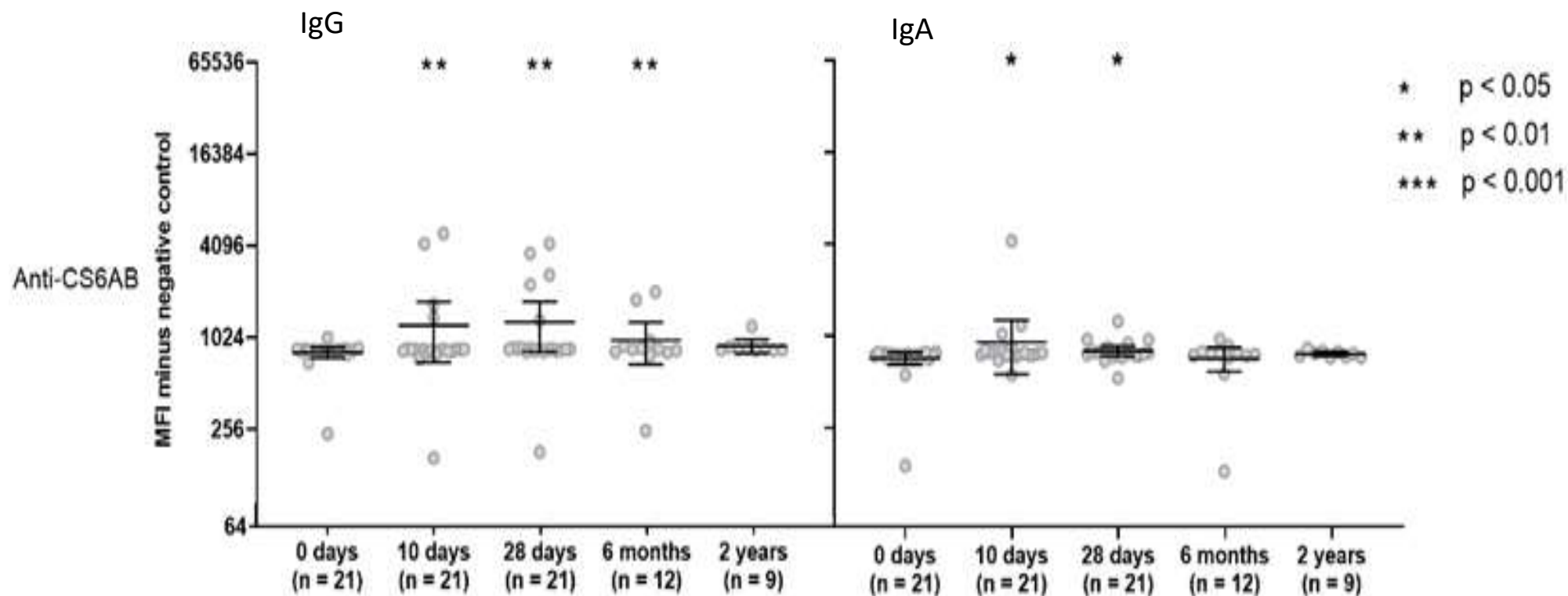
CI: 0.40 - 0.97

Symptom	Dose (CFU)	
	$1 \times 10^6 - 1 \times 10^9$ n = 12	1×10^{10} n = 9
Nausea	4 (33)	6 (67)
Abdominal pain	5 (42)	6 (67)
Abdominal cramping	2 (17)	5 (56)
Excessive flatus	4 (33)	7 (78)
Decreased appetite	1 (8)	2 (22)
Bloating	4 (33)	7 (78)
Vomiting	0 (0)	2 (22)
Constipation	0 (0)	1 (11)
Headache	4 (33)	5 (56)
Malaise	3 (33)	6 (67)
Fever	2 (17)	0 (0)
Chills	1 (8)	0 (0)
Generalized myalgias	1 (8)	0 (0)
Lightheadedness	3 (25)	2 (22)
Hypovolemia	0 (0)	0 (0)

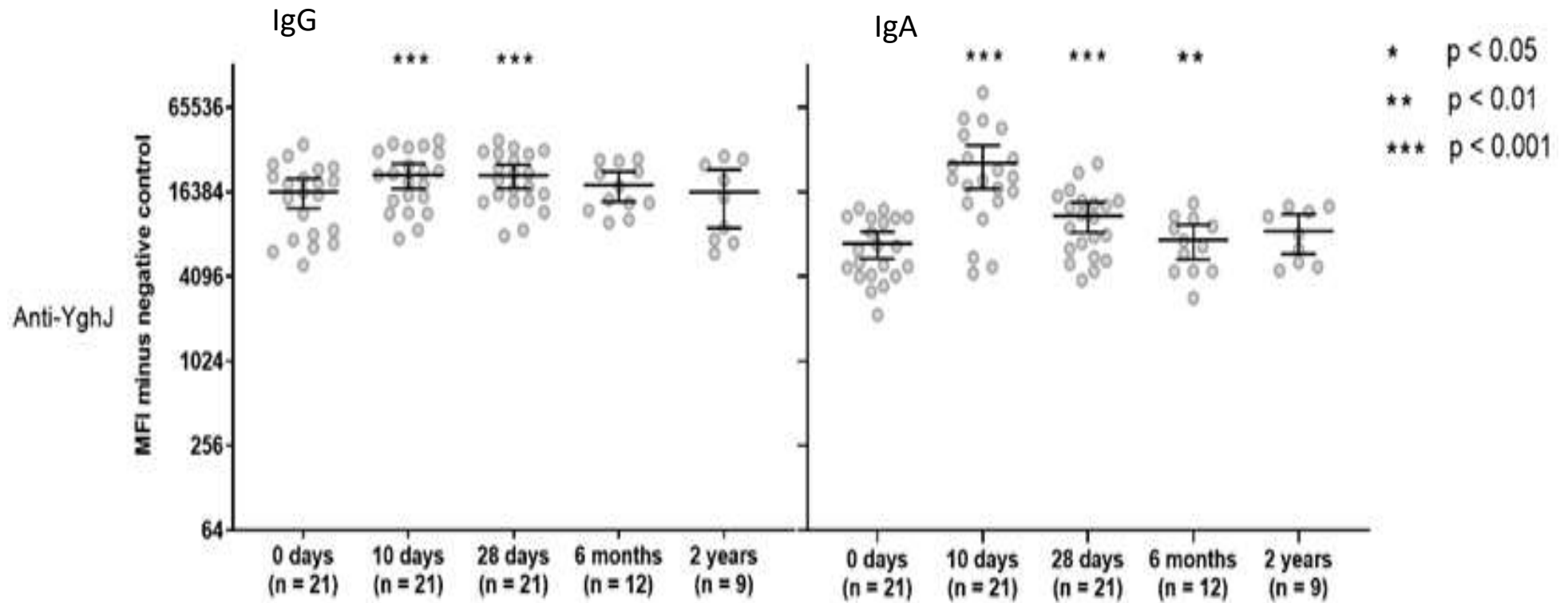
Serum anti-CS5 antibody responses TW10722



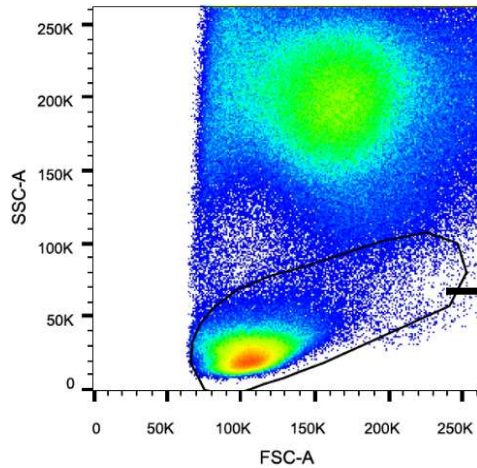
Serum anti-CS6AB antibody responses TW10722



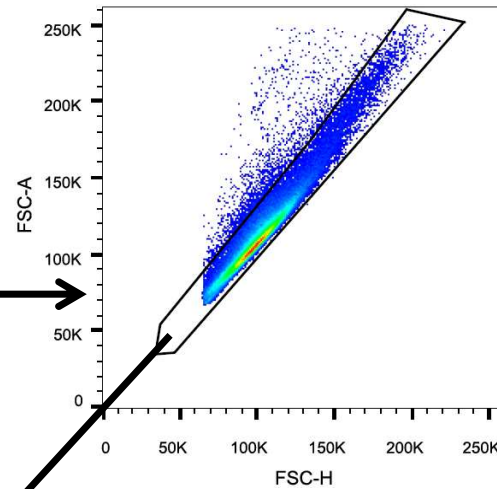
Serum anti-YghJ antibody responses TW10722



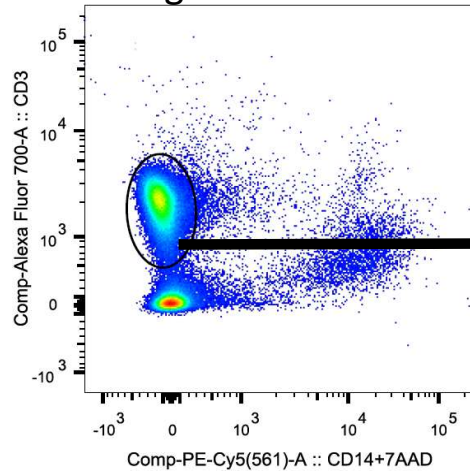
Cells after RBC lysis



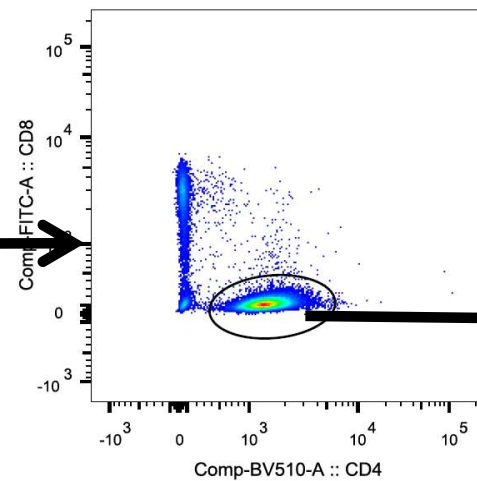
Mononuclear cells



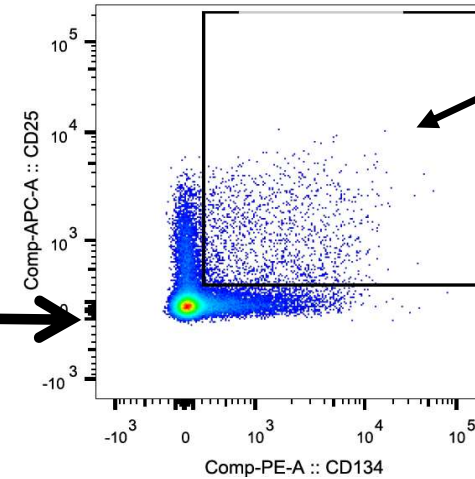
Single cells



Live CD3 T cells



CD4 T cells

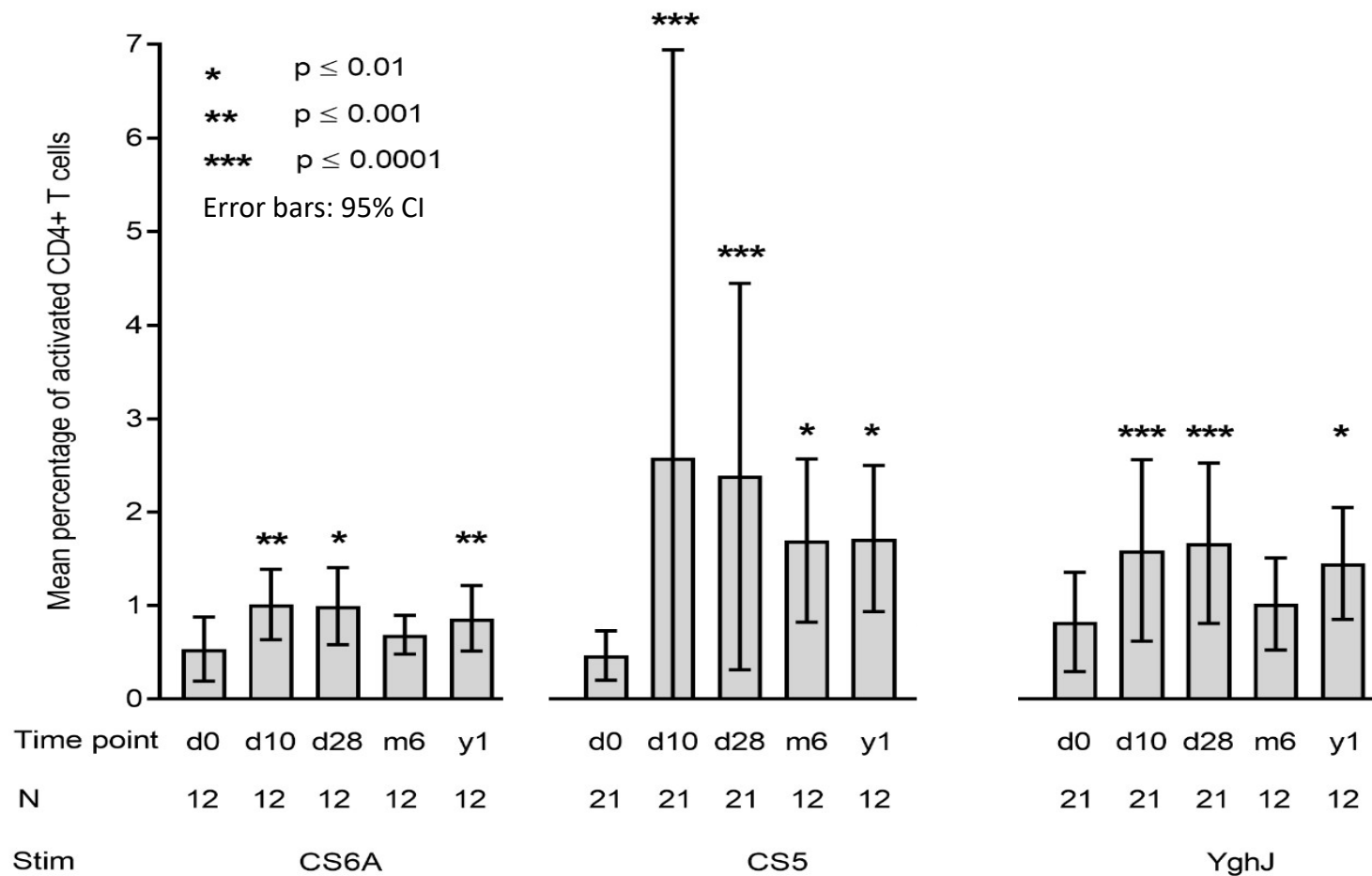


Percentage of activated CD4 T cells co-expressing CD25 and CD134 (OX-40).

Two day whole blood T cell assay



CD4+ T cell responses to TW10722 antigens



Correlations between antibody responses and CD4 T cell responses

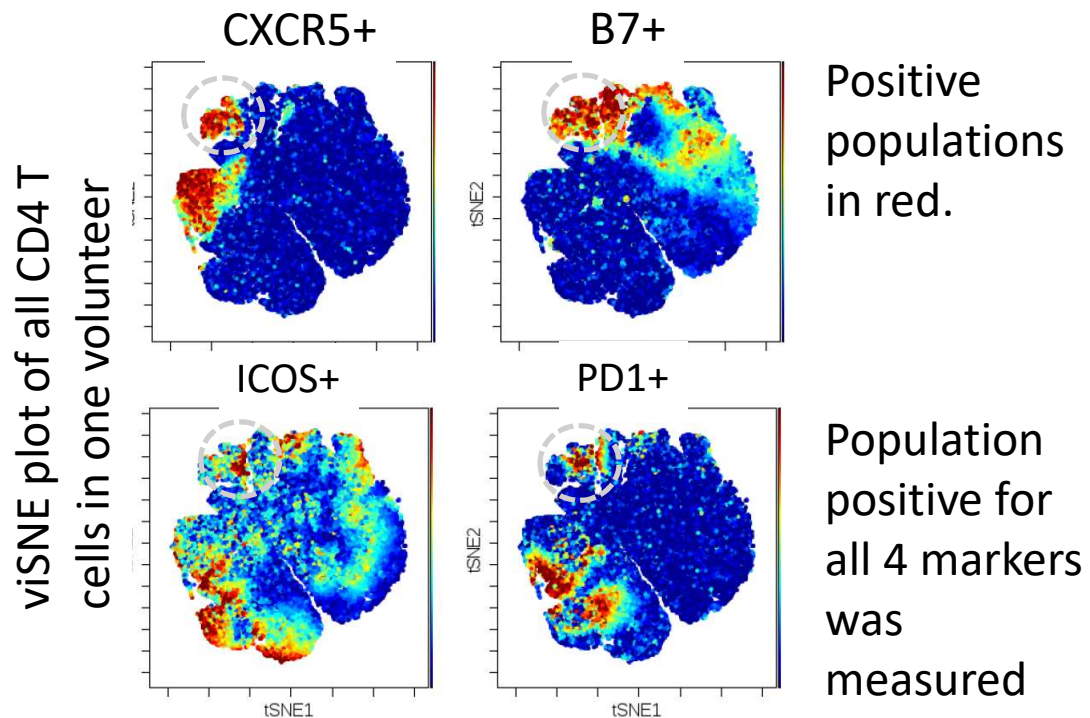
- Serum anti-CS5 ($p = 0.023$) and anti-CS6AB ($p = 0.048$) IgA antibody levels were associated with increases in the corresponding CD4+ T cell responses
- No significant association was found between YghJ-specific serum antibody and CD4 T cell responses.

Clinical symptoms and immune responses?

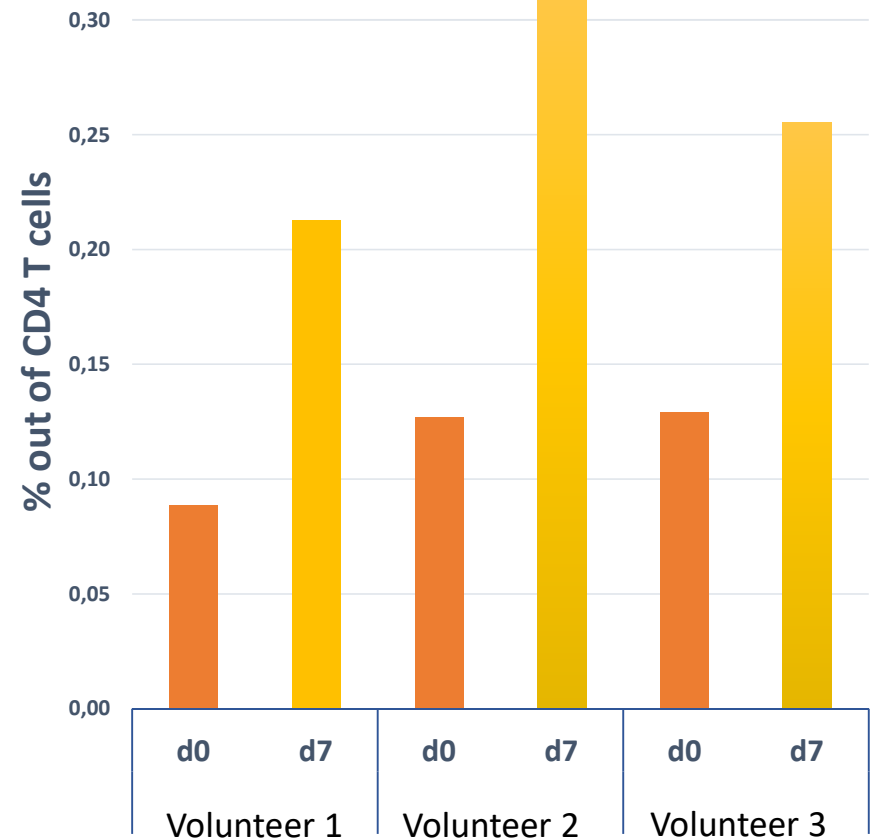
- **Development of diarrhea** was associated with
 - increase in anti-CS5 IgA ($p = 0.036$)
 - increase in YghJ-specific CD4+ T cells ($p = 0.033$)
- There was no association between **inoculation dose** and CS5, CS6, or YghJ-specific antibody or activated CD4+ T cell levels.

Piloting ways to evaluate correlation of protection

– circulating Tfh in three volunteers



Activated gut-homing cTfh cells (CXCR5+ B7+ ICOS+ PD1+)



Mean 2.5-fold increase from day 0 to day 7

Summary

STh-only ETEC strain TW10722:

- Safe
 - Attack risk 78% for diarrhea (1×10^{10} CFU).
 - Useful for testing ST-based vaccine candidates
 - More volunteers desirable for better attack risk estimate
-
- Experimental ETEC infection with TW10722 elicited rapid human CD4 T cell immune response against CS5 and mucinase YghJ, and CS6 in some.
 - The serological responses to colonization factors CS5 and CS6 were slower to develop than for mucinase YghJ, while T cell responses showed similar kinetics.

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Material in biobank

- PBMCs **d0, d7, d10, d28, 3m, 6m**
- Sera **d0, d10, d28, 3m, 6m**
- Plasma **d0, d1, d2, d3, d7**
- ALS **PBMCs cultured d0 and d7**
- Saliva **d0, d10, d28**
- Intestinal lavage **d0, d10**
- Stool specimens **d0-d10, d28, (6m)**