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Faculty of Veterinary Medicine

PROVAXS

Heat-labile enterotoxin enhances small intestinal colonization with F18ac+ verotoxigenic *E. coli* in newly weaned piglets



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Overall hypothesis of the study

LT enterotoxin in subclinical concentrations

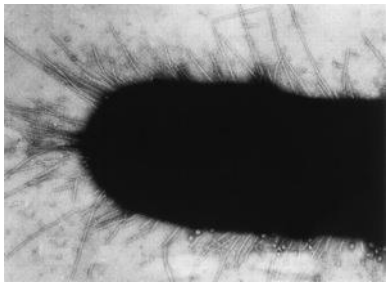
affects the small intestinal mucosa making it

more susceptible to other enteropathogens

- Pig as model
- Enterotoxigenic E. coli as source of LT

If this could be proven vaccination against LT would be a valid strategy

E. coli infections post weaning in piglets



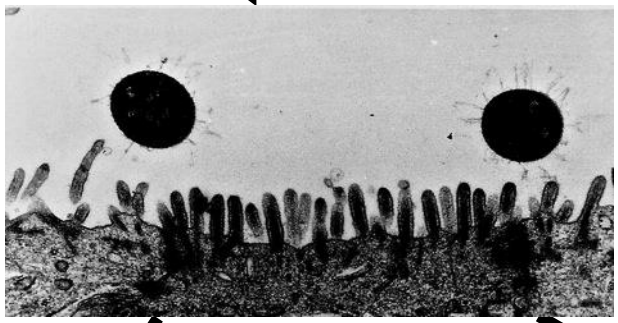
Enterotoxigenic E. coli (ETEC), F4 or F18 fimbriae

Vérotoxinogenic E. coli (VTEC), F18 fimbriae

↓

Fimbriae (F4, F18)

↓



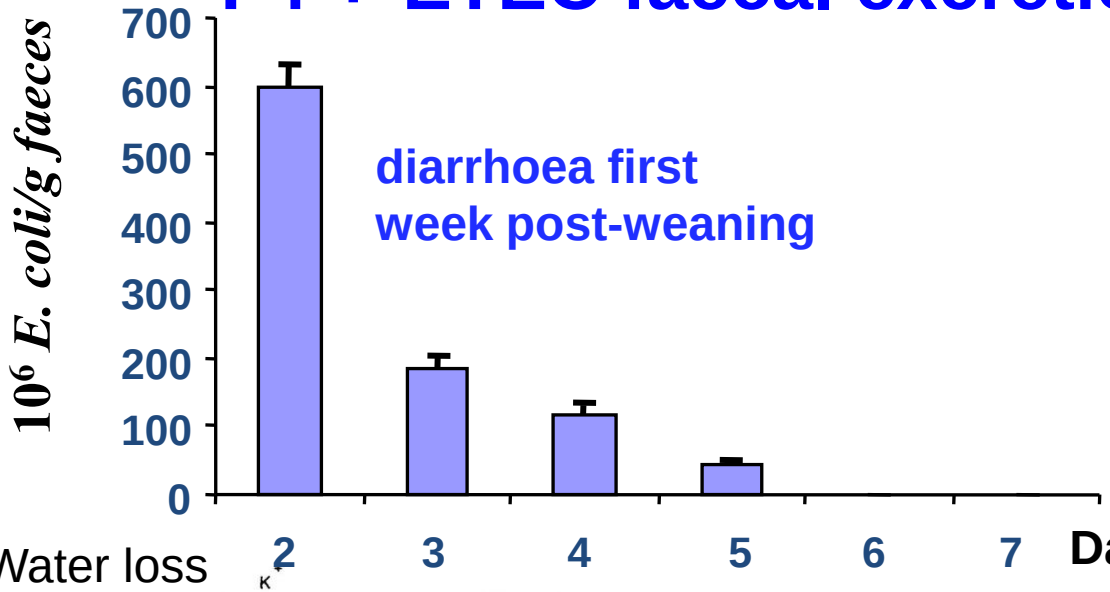
colonisation

↓ Enterotoxins (LT-I, STa, STb) → Diarrhoea

↓ Shiga-toxin (Stx2e) → Oedema disease

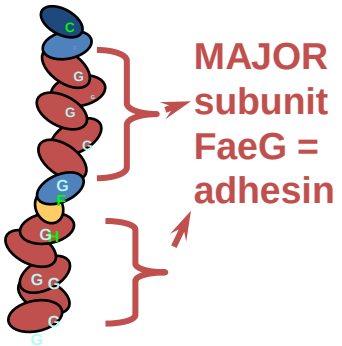
Hypothesis: Can an F4 ETEC infection enhance an F18 VTEC infection

F4 + ETEC faecal excretion

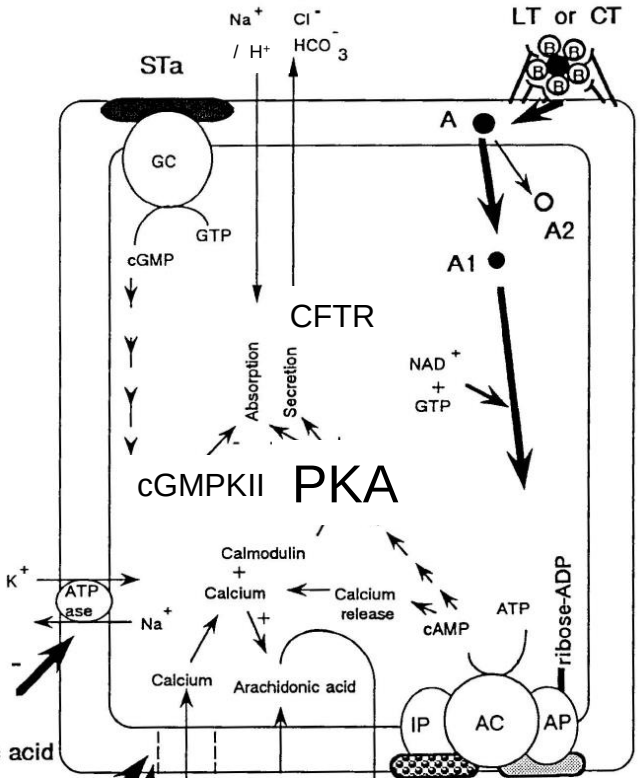


Water loss

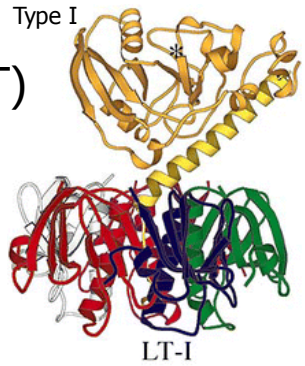
F4 fimbriae



Enterotoxins
(LT-I, STa, STb, EAST1)



Thermo-labile enterotoxin (LT)
=AB5 toxin
Binds to GM1Ganglioside on enterocytes

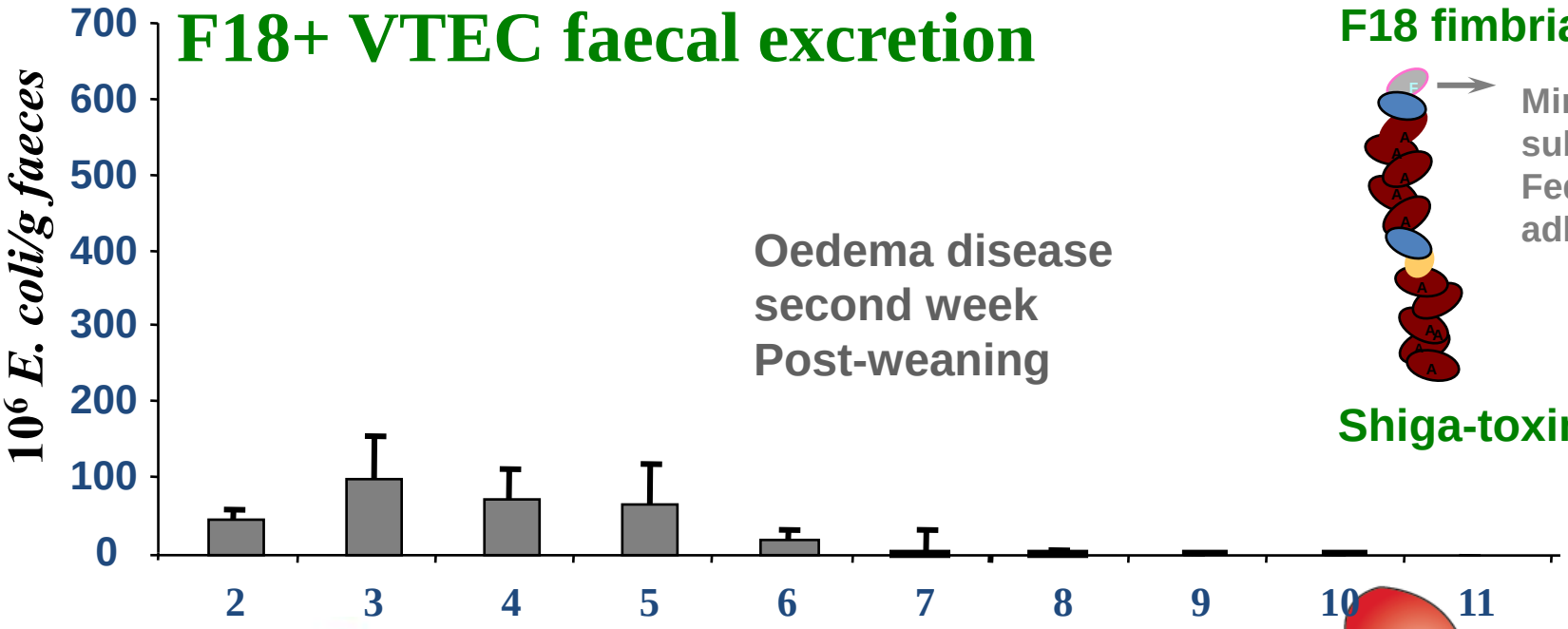


CFTR= cystic fibrosis transmembrane regulator

PKA= protein kinase A

Enterocyte

F18+ VTEC faecal excretion

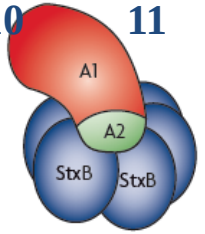


Oedema disease
second week
Post-weaning

F18 fimbriae

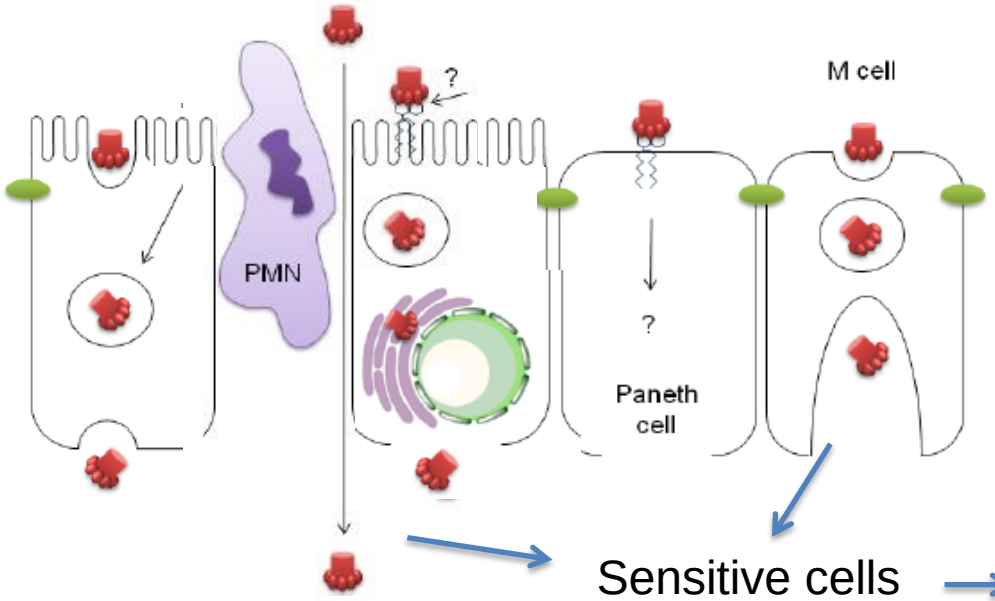
Minor subunit FedF = adhesin

Shiga-toxin (Stx2e)



Stx

Binds to Gb3

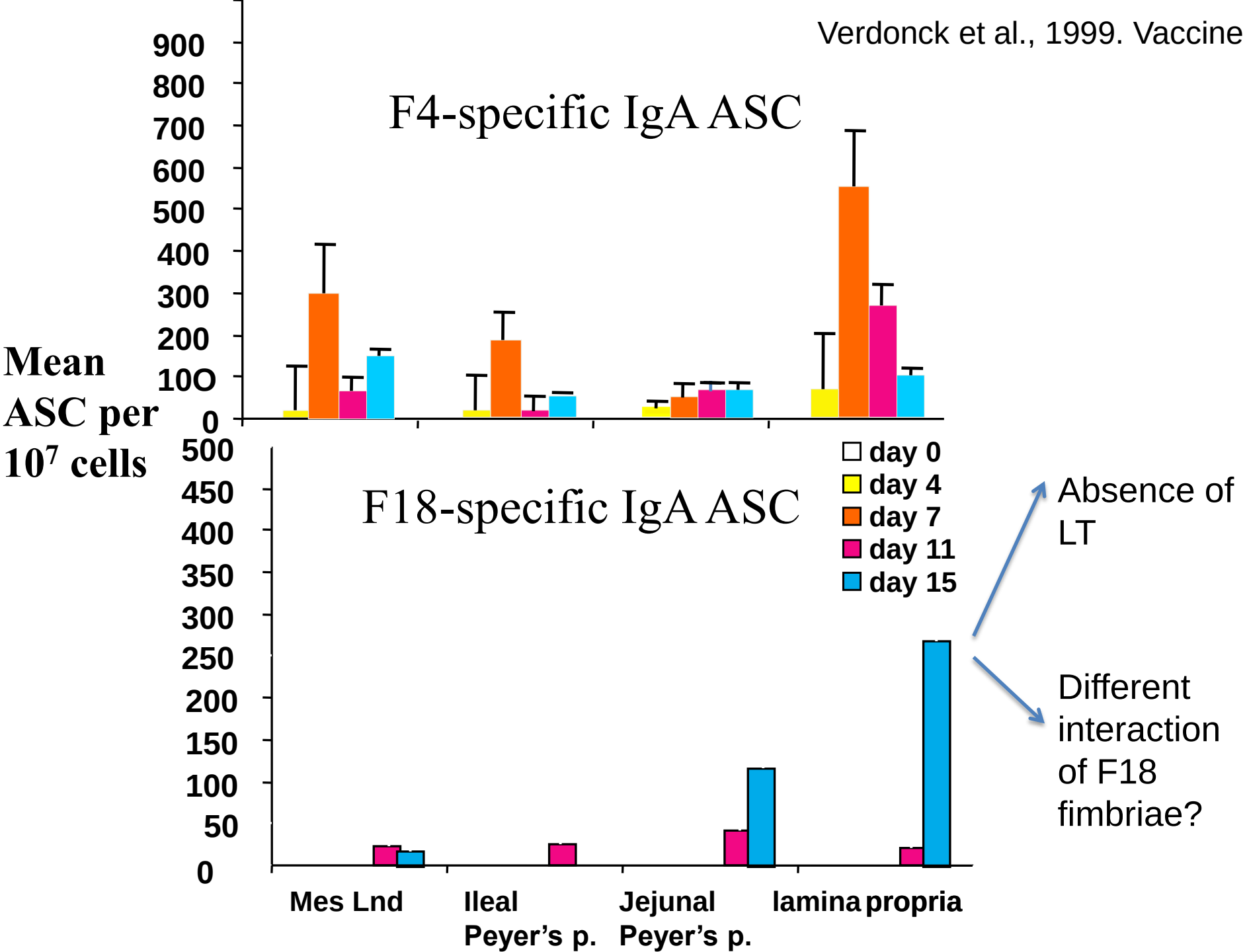


Intestinal epithelium

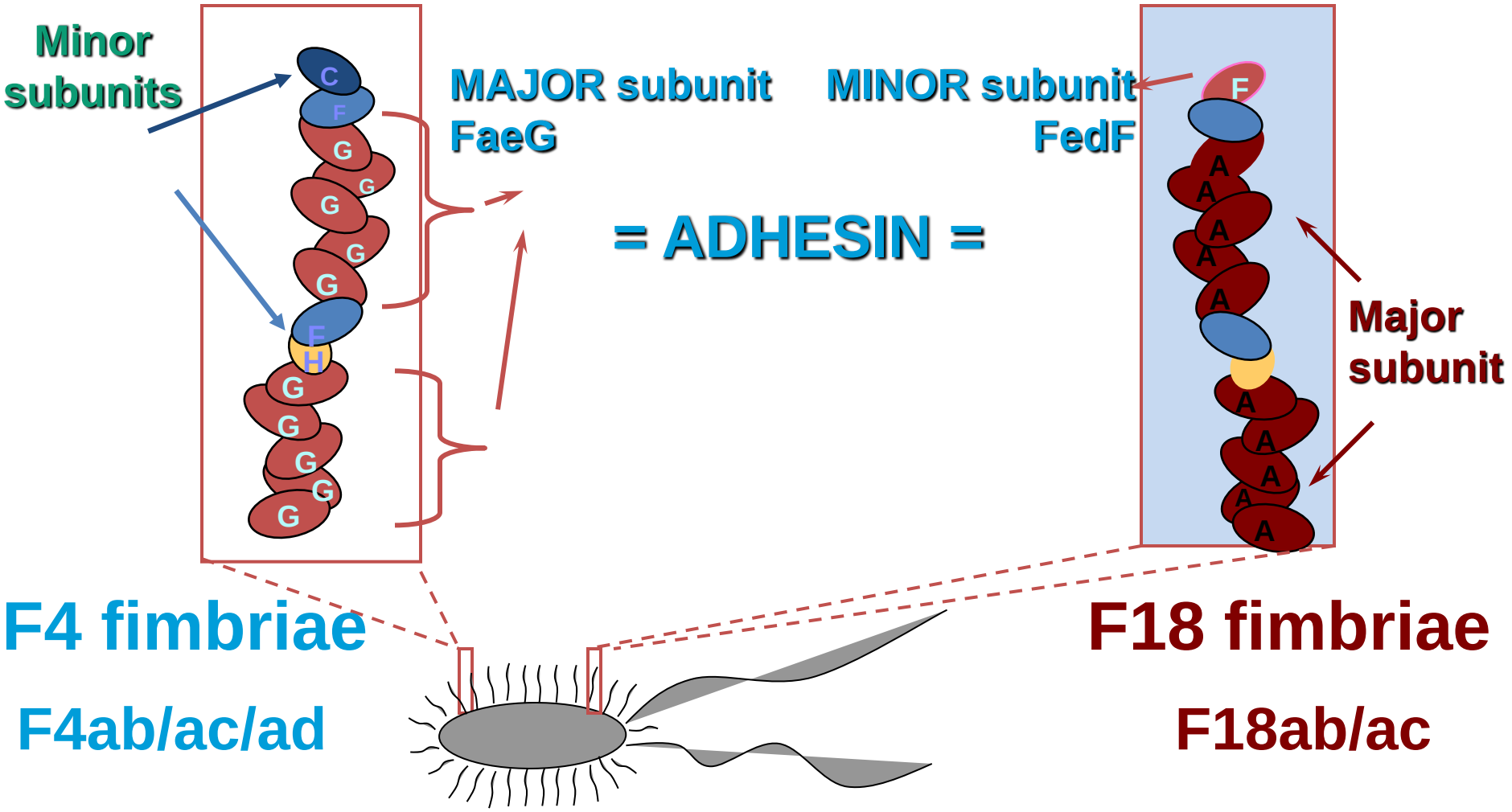
Sensitive cells → Apoptosis endothelial cells → oedema

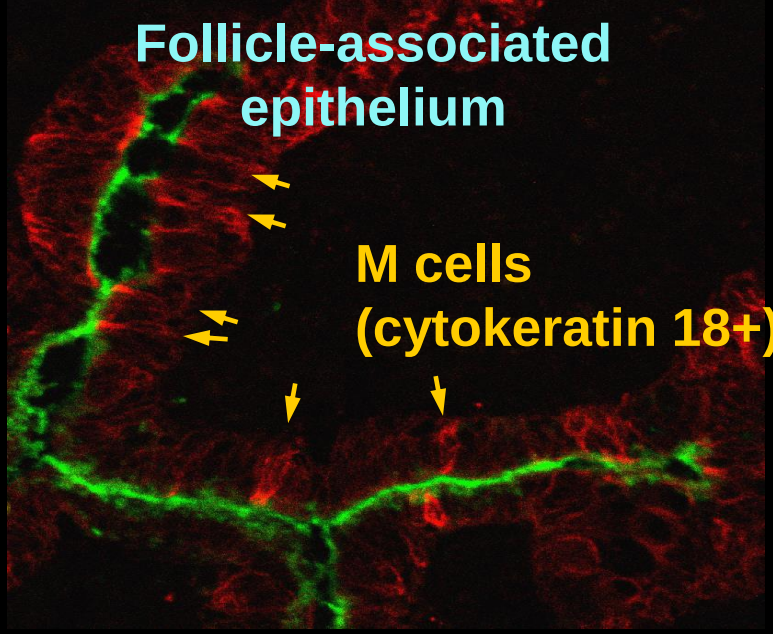
Johannes and Römer, 2010. Nature Review Microbiology; Schüler, 2011. Toxins

Verdonck et al., 1999. Vaccine



F4 and F18

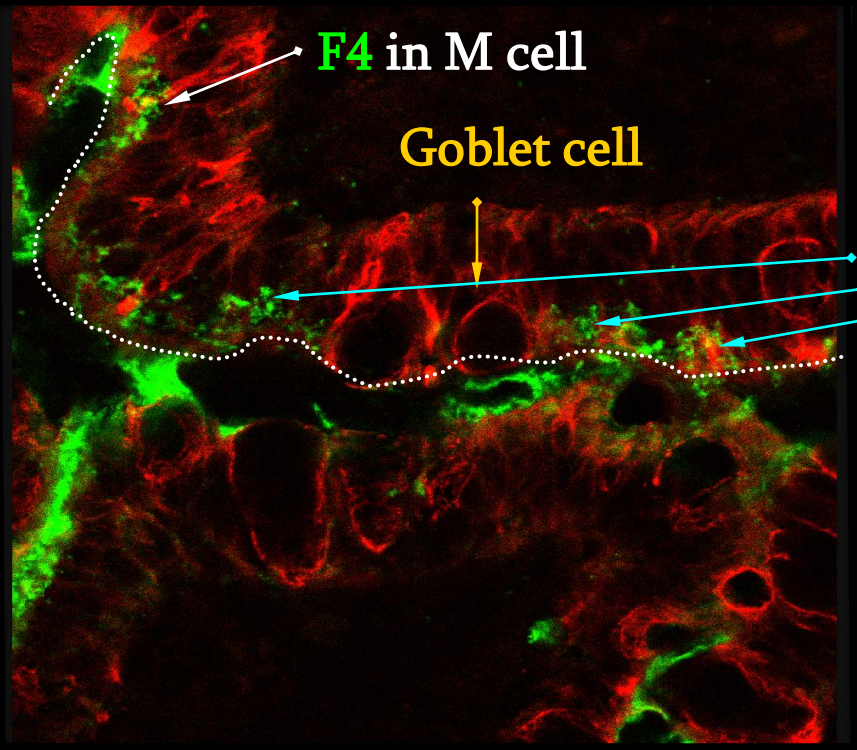

Minor subunits
C
F
G
G
G
G
G
G
F
H
G
G
G
G
G
MAJOR subunit
FaeG
MINOR subunit
FedF
= ADHESIN =
F
A
A
A
A
A
A
A
A
A
A
Major subunit
F4 fimbriae
F4ab/ac/ad
F18 fimbriae
F18ab/ac



Binding and uptake of F4

Ligated loops injected with F4



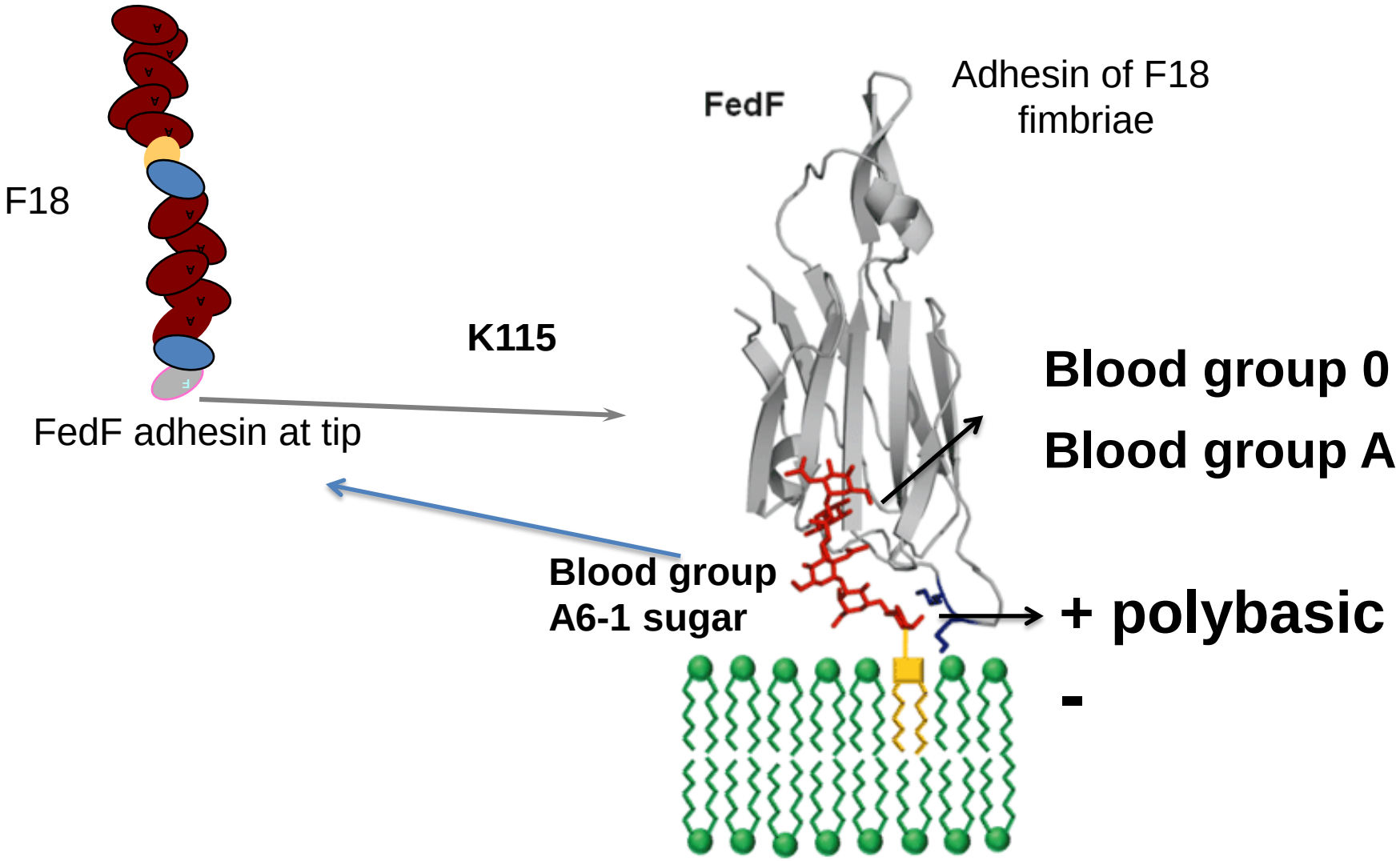


Cytokeratin-18 18

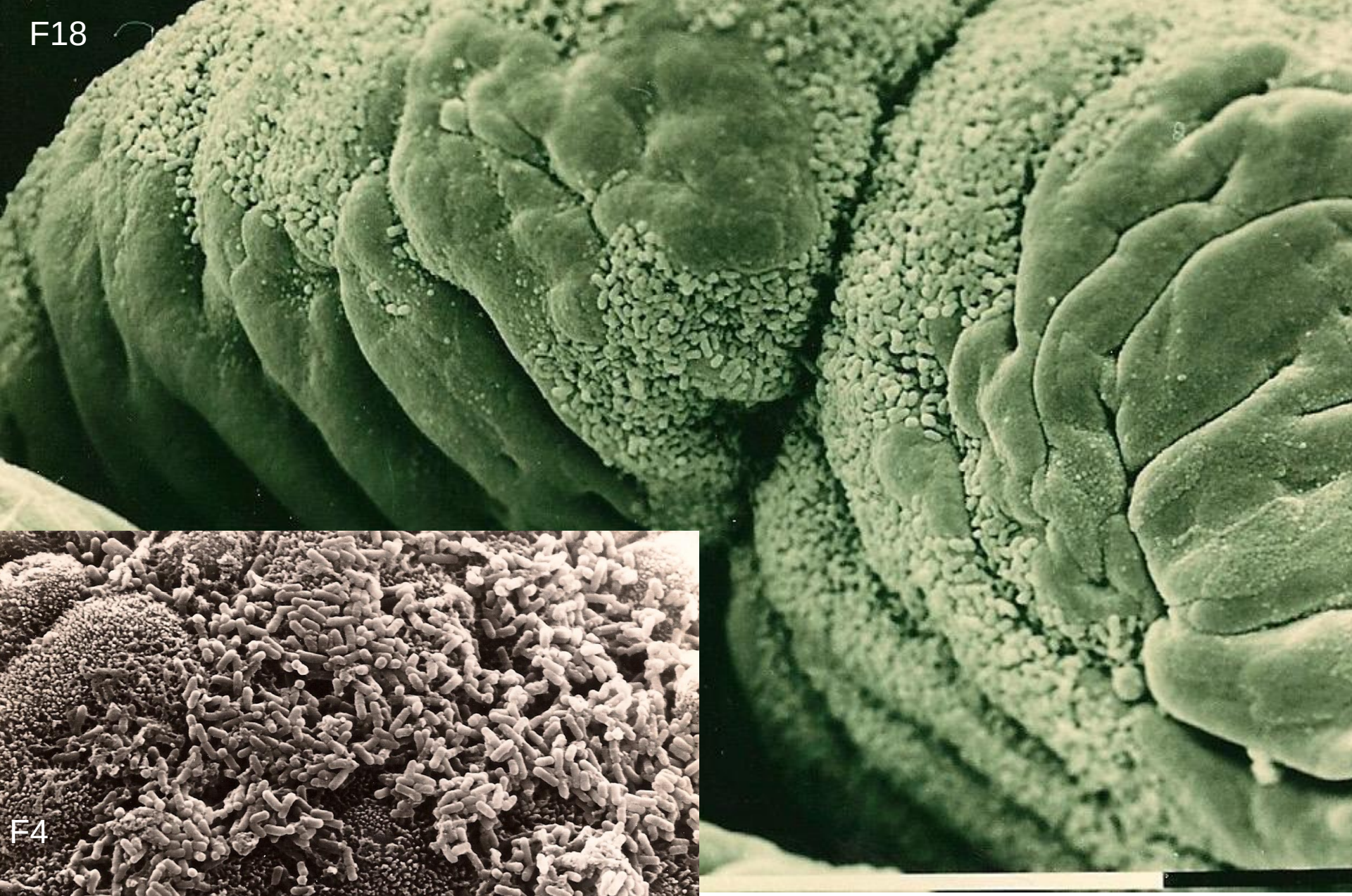
F4 fimbriae

Snoeck et al., 2008. Vet Imm Immunopath.

Structural insight in binding of blood group A6-1 sugar by the F18 fimbrial adhesin FedF



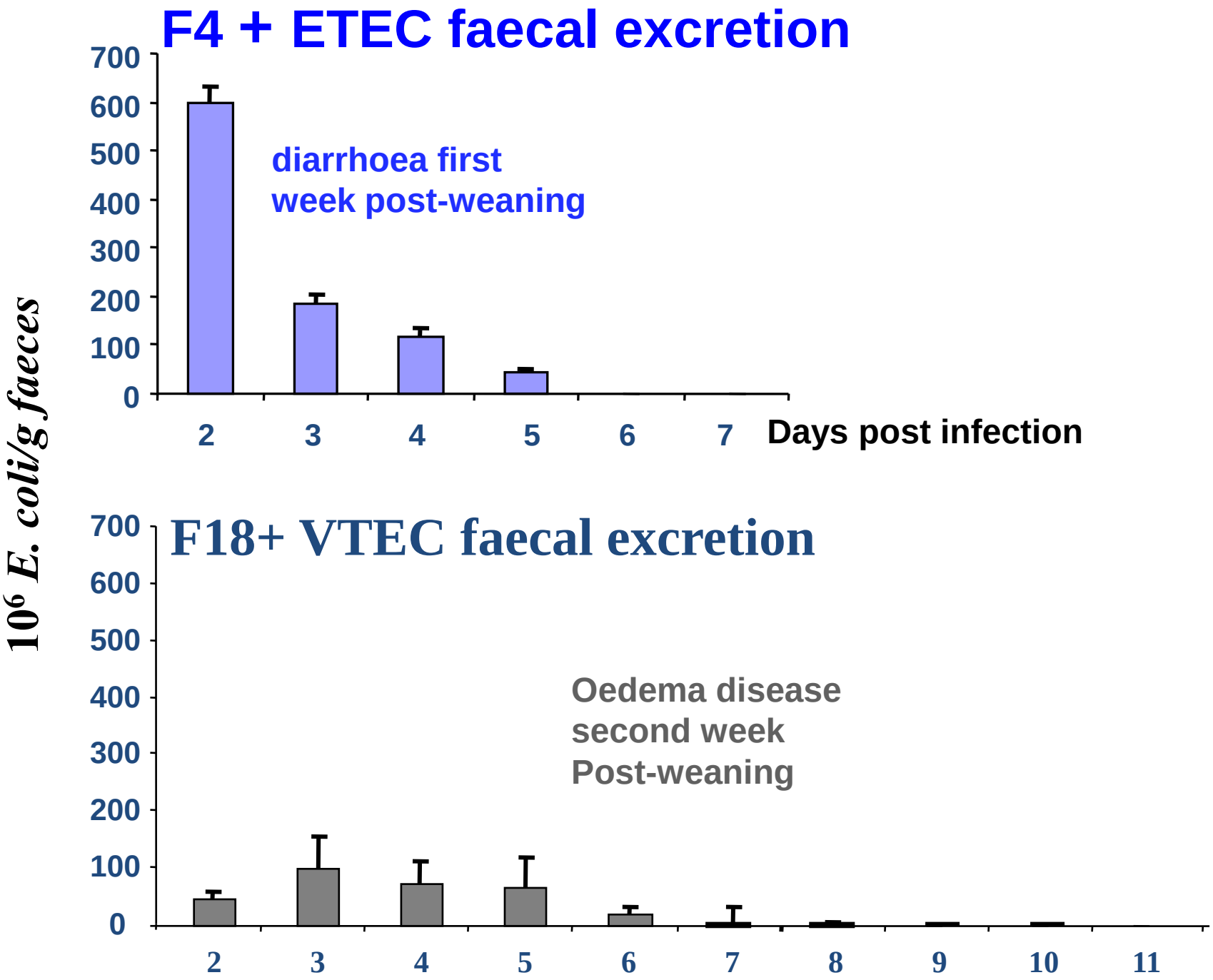
Moonens et al., Molecular Microbiology, 86, 82-95.


F18

F4

Hypothesis

Can LT enterotoxin in subclinical concentrations affect the small intestinal mucosa of newly weaned piglets so that it enhances F18 VTEC colonization ?



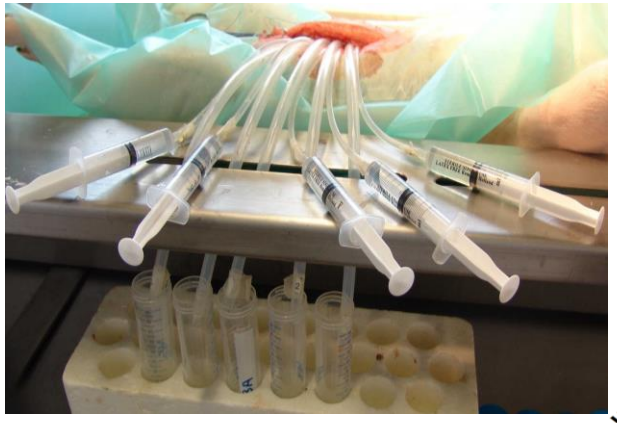
Verdonck et al., 2002. Vaccine

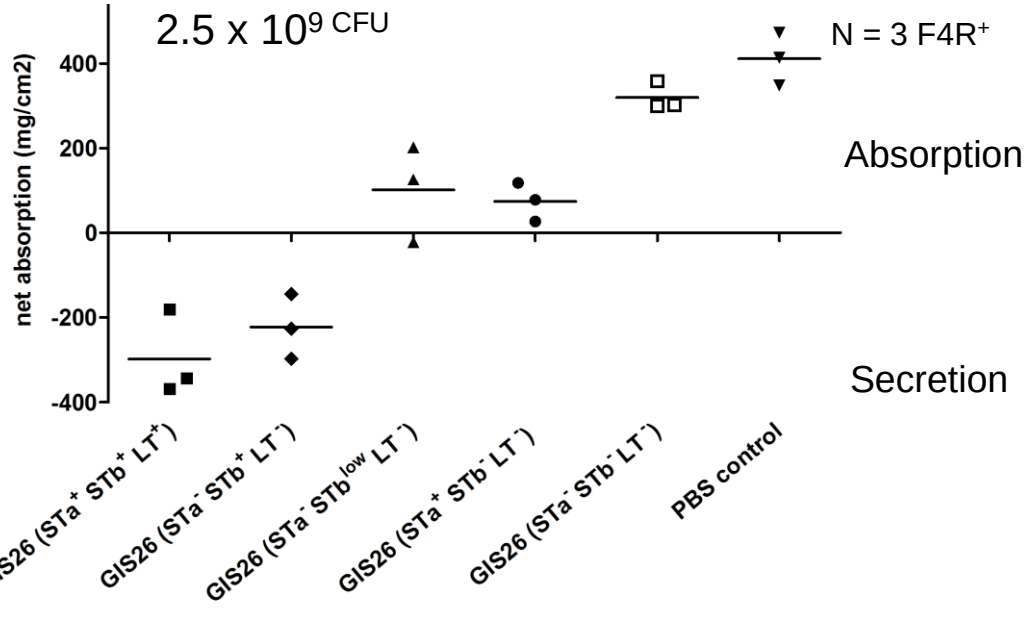
SMALL INTESTINAL SEGMENT PERFUSION (SISP)

GIS26 (O149:K91:F4ac, LT$^+$ STb $^+$Sta+)

F4R$^+$ pig 4h perfusion

20 cm segment

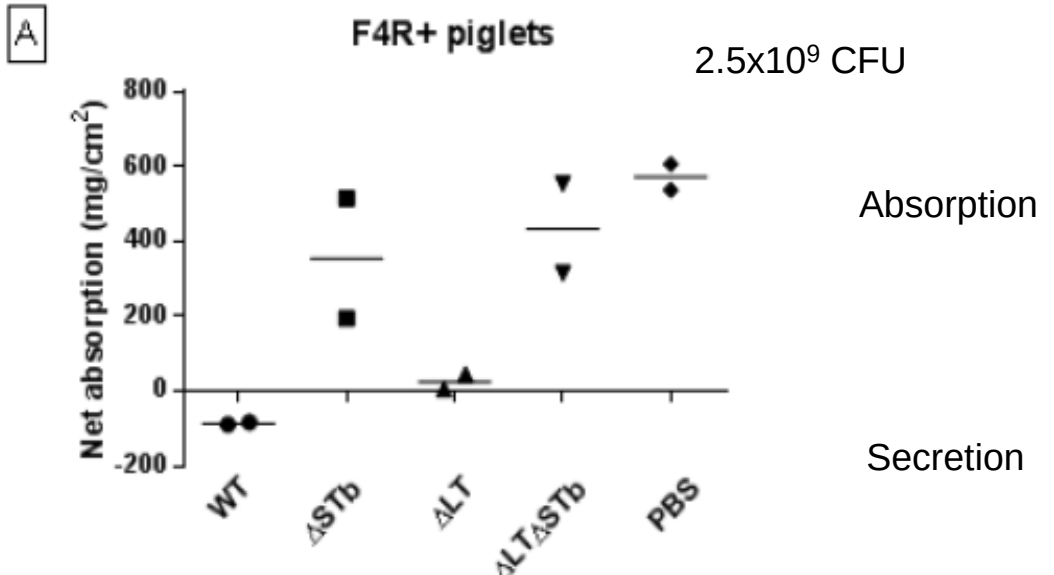




F4R$^+$ pig 7h perfusion

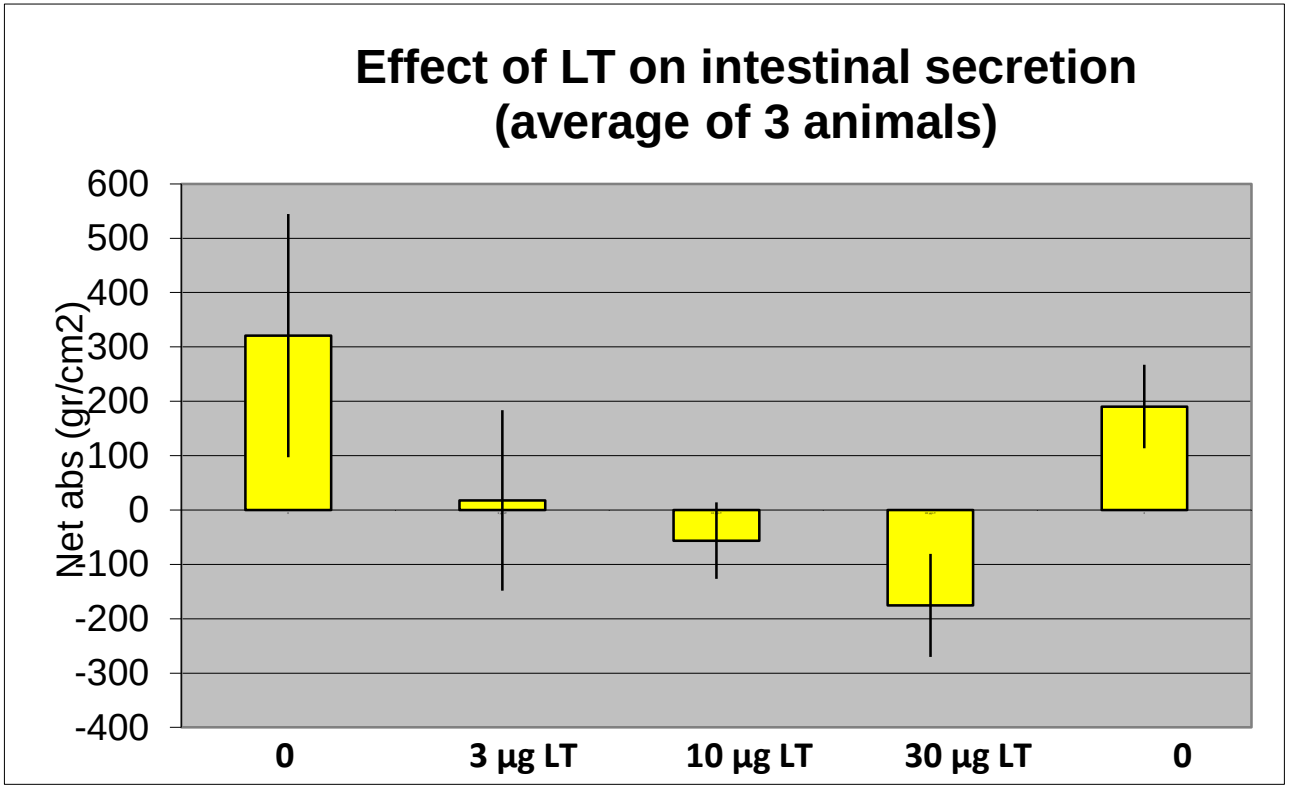
IMM01 (O149:K91:F4ac, LT$^+$ STb$^+$)

F4+ ETEC (Log10)	% Mucus coverage	SD
Control	89	3
4	84	7
6	73	8
8	68	8



Loos et al., 2012. PLoS ONE 7(7): e41041; Loos et al., unpublished data

What dosis for LT enterotoxin in the SISP of 20 cm?



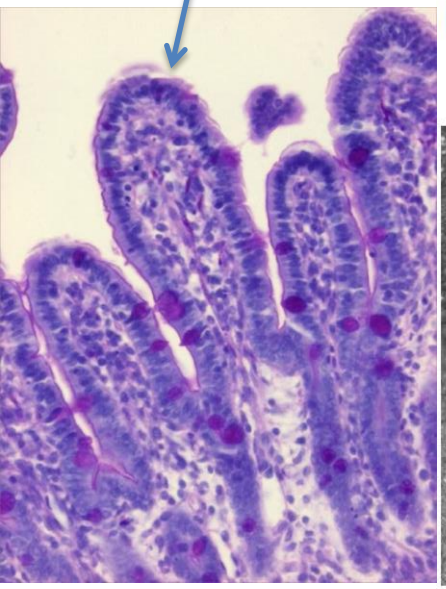
After 6 hours perfusion 10 µg was the lowest dose with Consistent fluid secretion

56 ± 70 g fluid per cm^2 mucosa

What is the effect of these dosages on mucus?

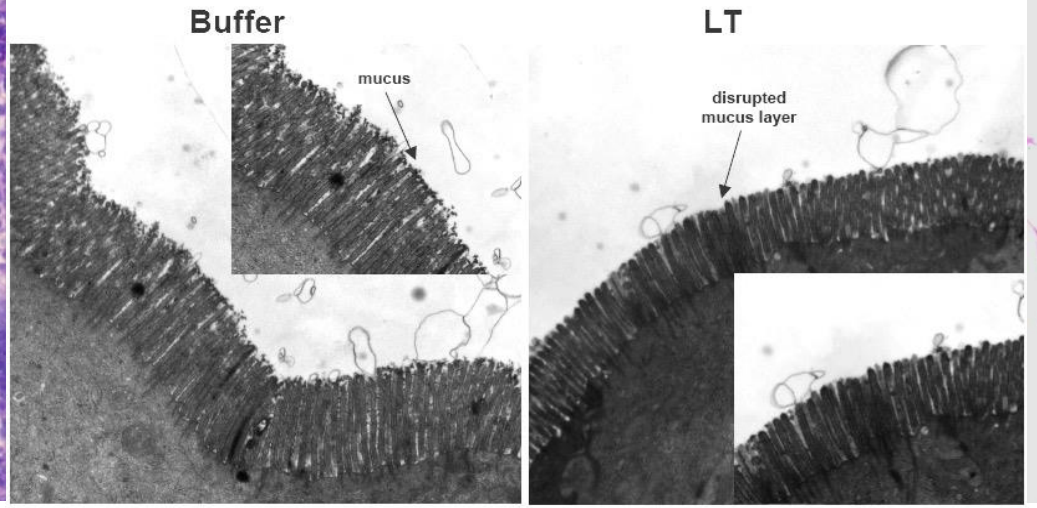
LT dose	Number of loops tested	% mucus coverage
0 µg	8	89 ± 5%
3 µg	4	84 ± 4%
10 µg	3	76 ± 7%
30 µg	3	60 ± 7%

Normal



PAS staining

TEM showing disruption of mucus layer after LT exposure in continuous flow intestinal loop model

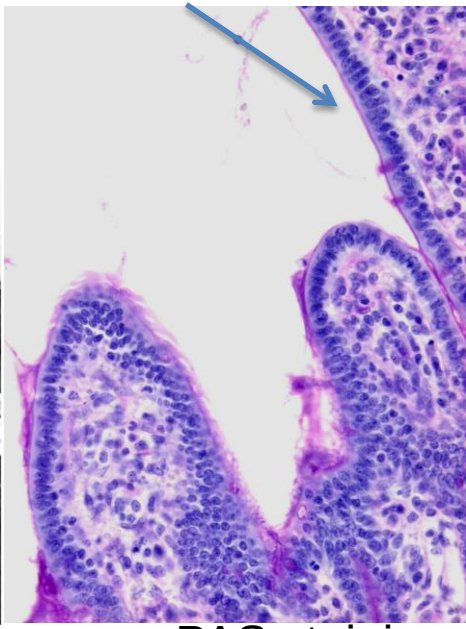


EM

Control

EM

Thin



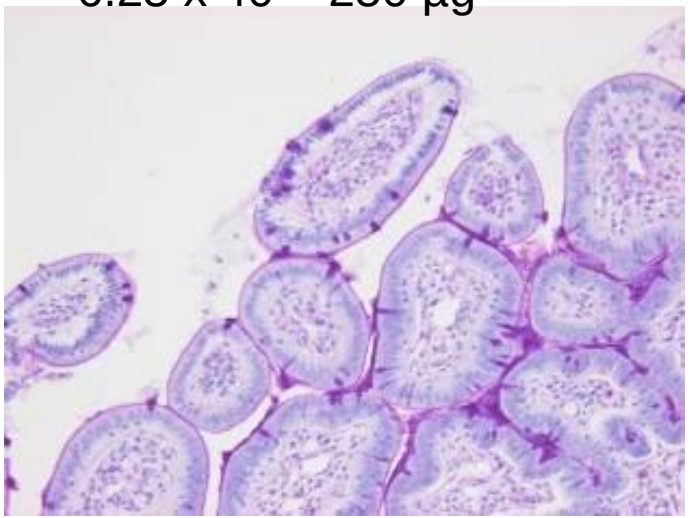
PAS staining

30 µg LT /ml

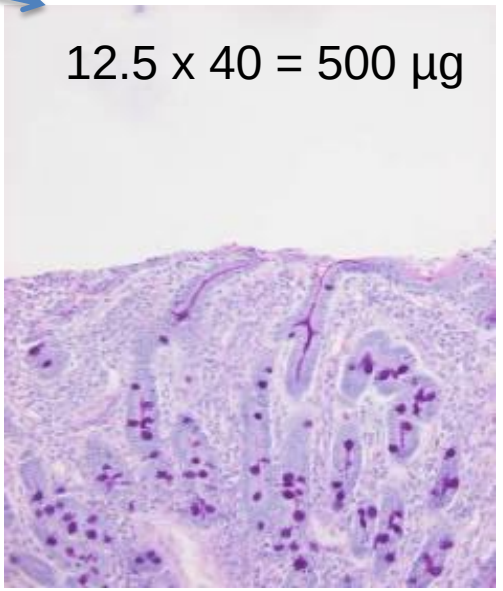
From 20 cm intestinal segments to 8 m intestine in pigs (injection at 5 sites)

Pig	BW (kg)	LT (µg)/animal	Euthanasia (hours after LT injection)	Symptoms	Histology
2	8	250	6	Normal intestinal contents at cecum and rectum	Normal
1	8	500	6	Watery intestinal contents at cecum and rectum	Removal of mucus
3	9	250	24	Normal feces and some fluid in jejunum	Normal
4	10	350	24	Soft feces and Fluid in ileum and jejunum	Normal
5	10	500	24	Diarrhoea	Removal of mucus

6.25 x 40 = 250 µg



12.5 x 40 = 500 µg



Conclusions

- Intra-intestinal injection of 500 µg LT induces diarrhoea in 4-week-old pigs.

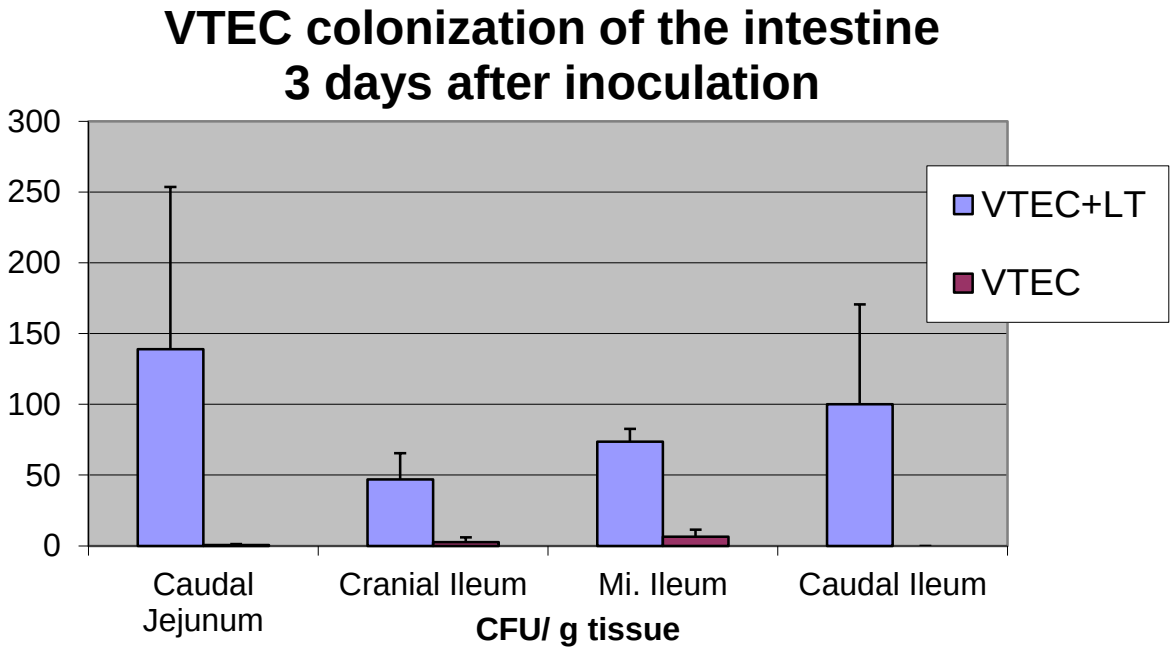
- 250 µg LT did not induce diarrhoea.

- Removal of mucus layer was only clearly detectable in animals which developed diarrhoea.

What is the effect on VTEC colonization

6 hours later

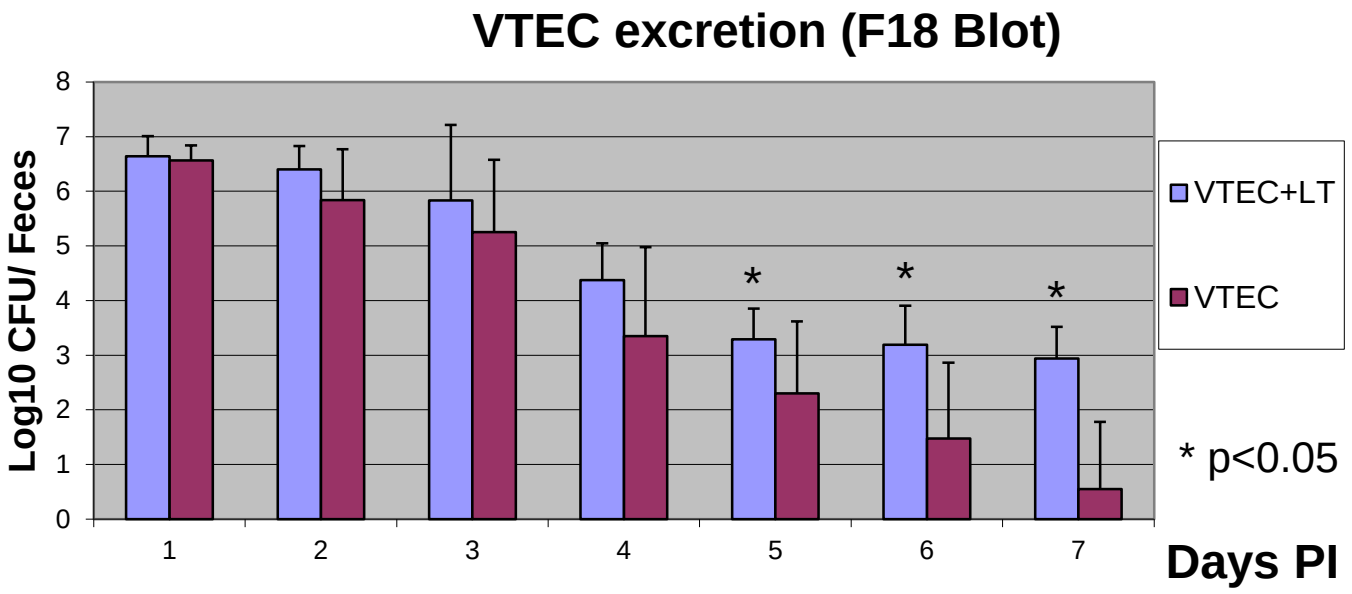
LT	F18ab+ VTEC	Pig	
250 µg	10**11 CFU in 10 ml PBS	1	
		2	
		3	
		4	† day 3
		5	† day 3
		6	† day 1
		7	† day 1
350 µg		8	
		9	
		10	
		11	† day 3
0 µg		12	
		13	
		14	
		15	
		16	
		17	† day 3
		18	† day 3
250 µg	-	19	† 6 hours
PBS		20	

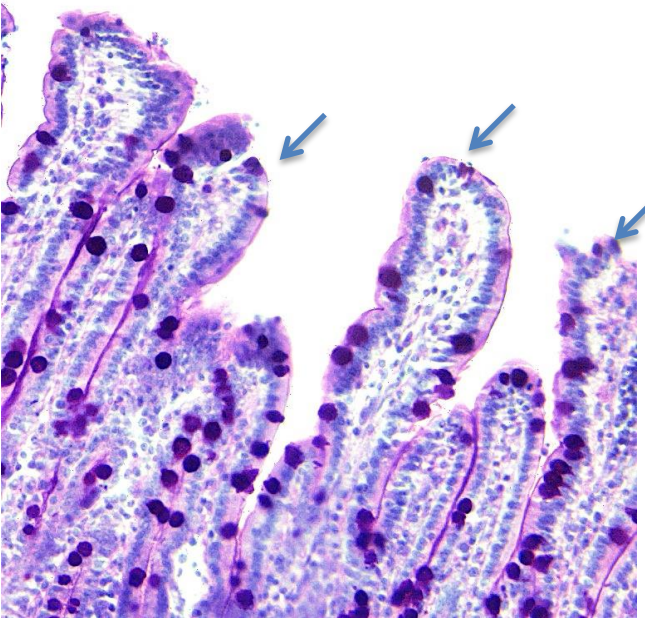


Mean duration

VTEC +LT: 7.0 ± 0.0 days*

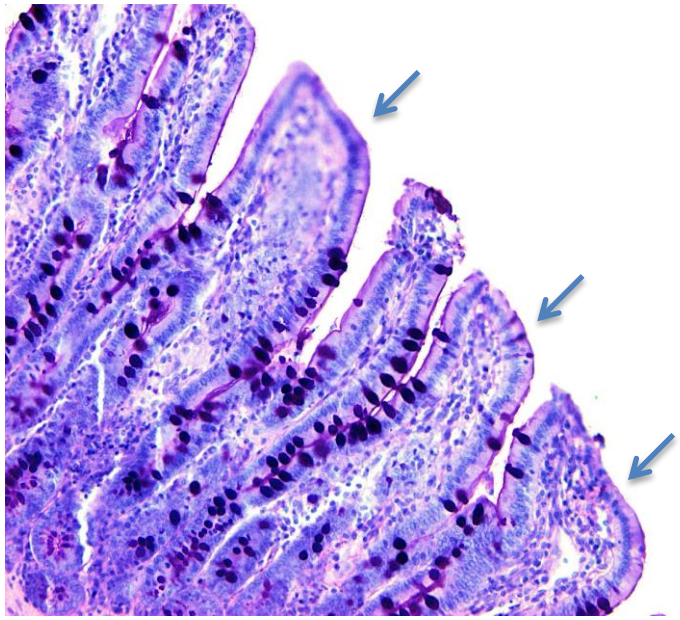
VTEC: 5.4 ± 1.4 days



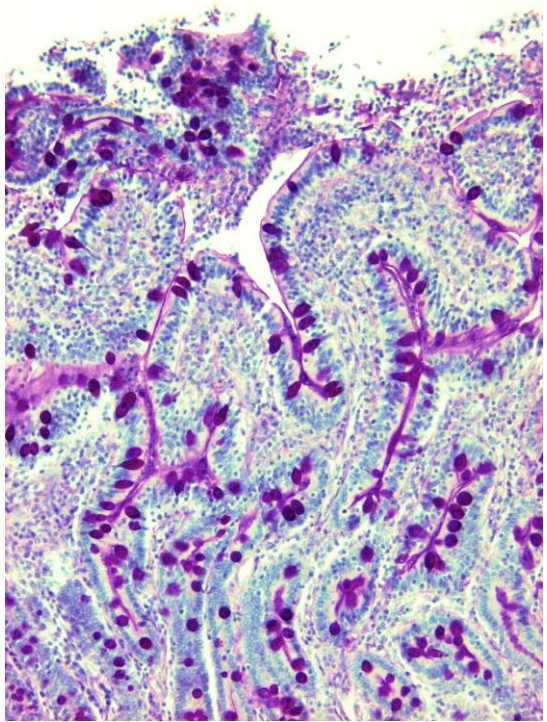


Pig 19
250 µg LT 6 hours

Mucus
thin at tips



Pig 7
250 µg LT + VTEC 4 hours



Pig 6
PBS

Conclusions

- Intra-intestinal injection of LT (250 or 350 µg) enhanced colonization with VTEC
- F4+ ETEC producing LT could play a role in colonization of the intestine with VTEC

Acknowledgements

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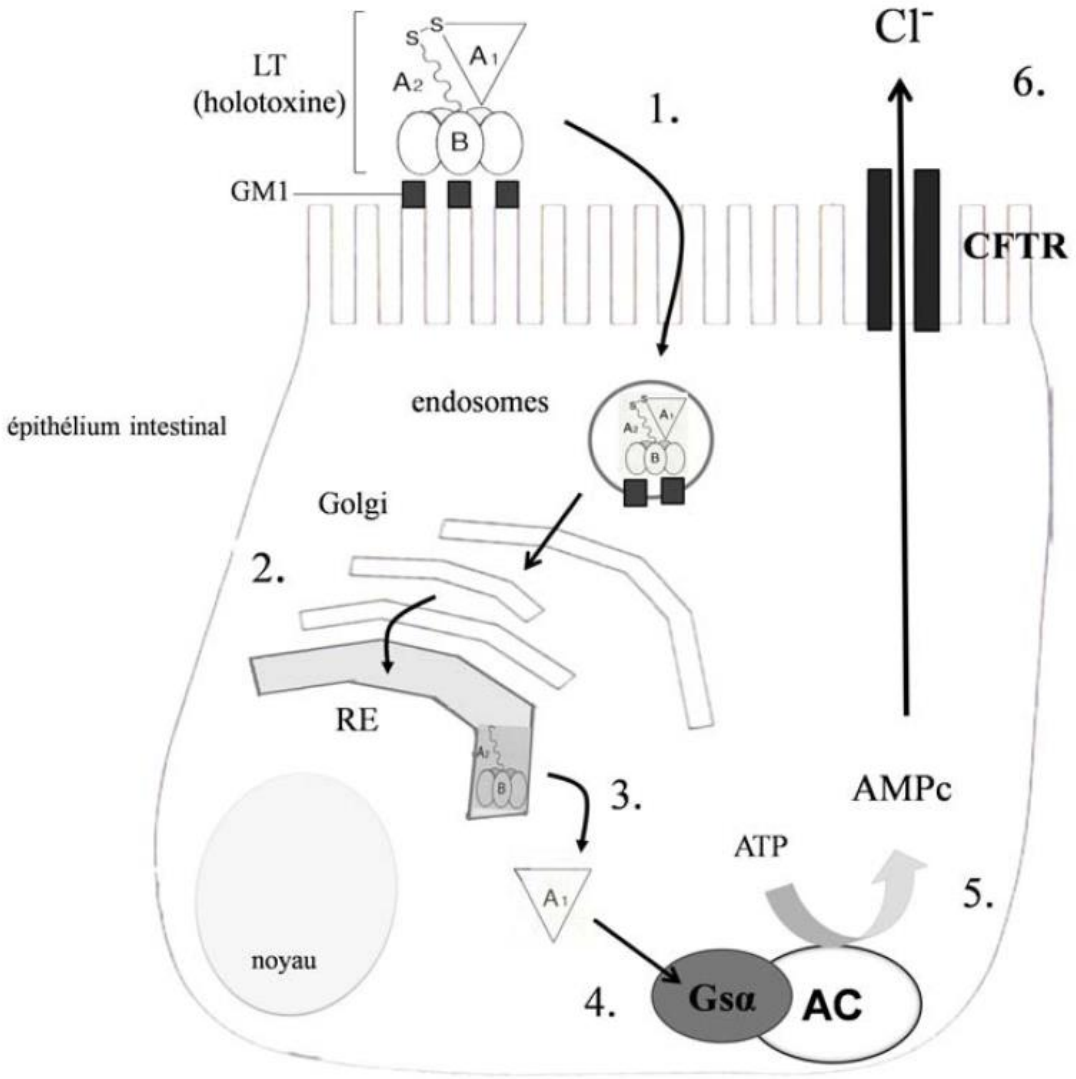
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Mechanism of action of LT-I



Hirst & D'Souza, 2006, Masignani et al., 2006

Toxin	Receptor	Internalization	Intracellular pathway	Target	Cellular alteration
LTI and CT	GM1	Receptor mediated endocytosis	Retrograde transport to Golgi → ER	Protein Gs	ATP → cAMP Adenylate cyclase
STa	Guanylate cyclase	No	X	Activation of catalytic domain of GC-C	GTP → cGTP Guanylate cyclase
STb	Sulfatide	Yes (Receptor mediated endocytosis?)	Unknown	Protein G	↑Ca^{++} Phospholipases activation

Protein kinase A => phosphorylation

cGMP-dependent protein kinase II => **CFTR** cystic fibrosis transmembrane regulator

Protein kinase C => phosphorylation

Secretion of
- **Chloride**
- **Carbonate**

Inhibition of
- **Sodium**

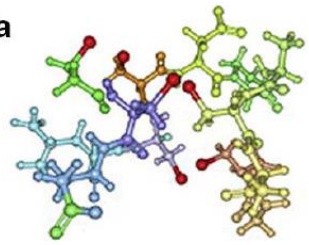
Osmosis-driven water secretion

Secretory diarrhea

Adapted from Dubreuil JD, 2013. Toxins

LT enterotoxin
Sta enterotoxin

STa

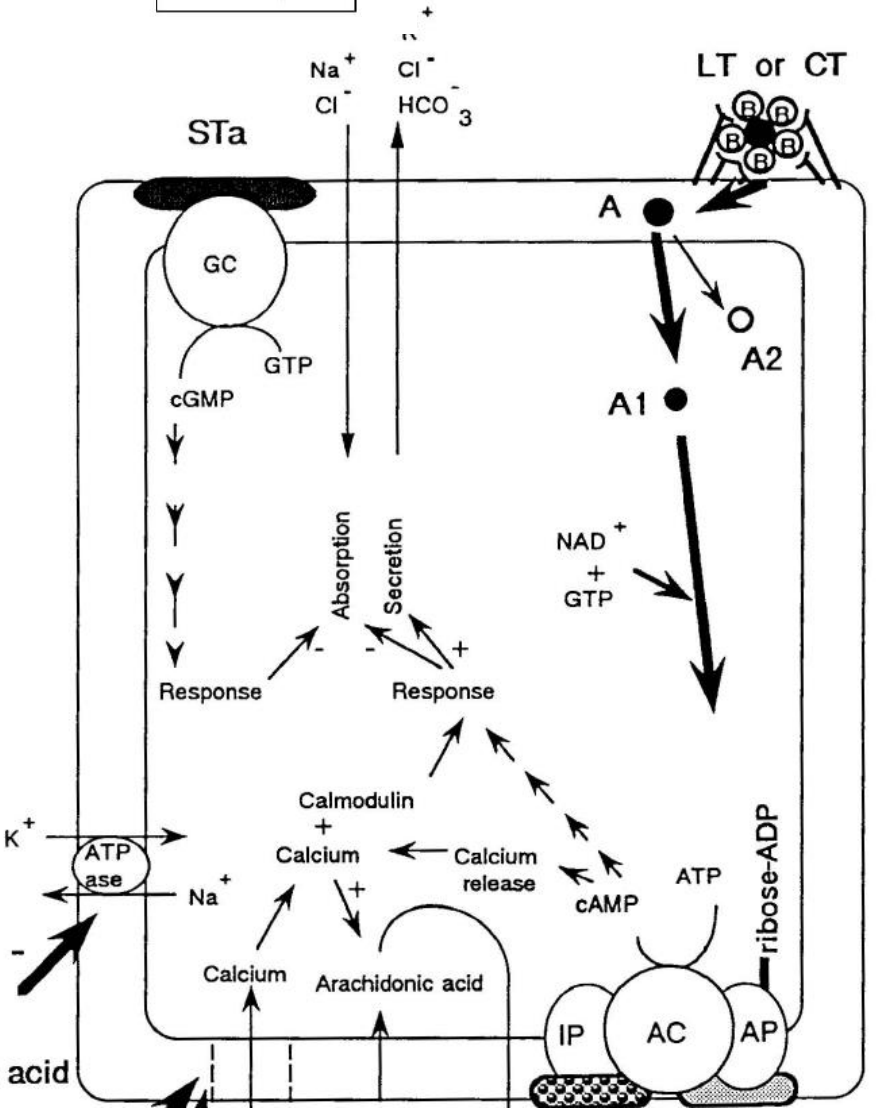


STaH: N S S N Y C C E L C C N P A C T G C Y

STaP: N T F Y C C E L C C N P A C A G C Y

Type I





Enterocyte