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Faculty of Veterinary Medicine

PROVAXS

Interaction *of E. coli* with the intestinal immune system of the pig in the post-weaning period

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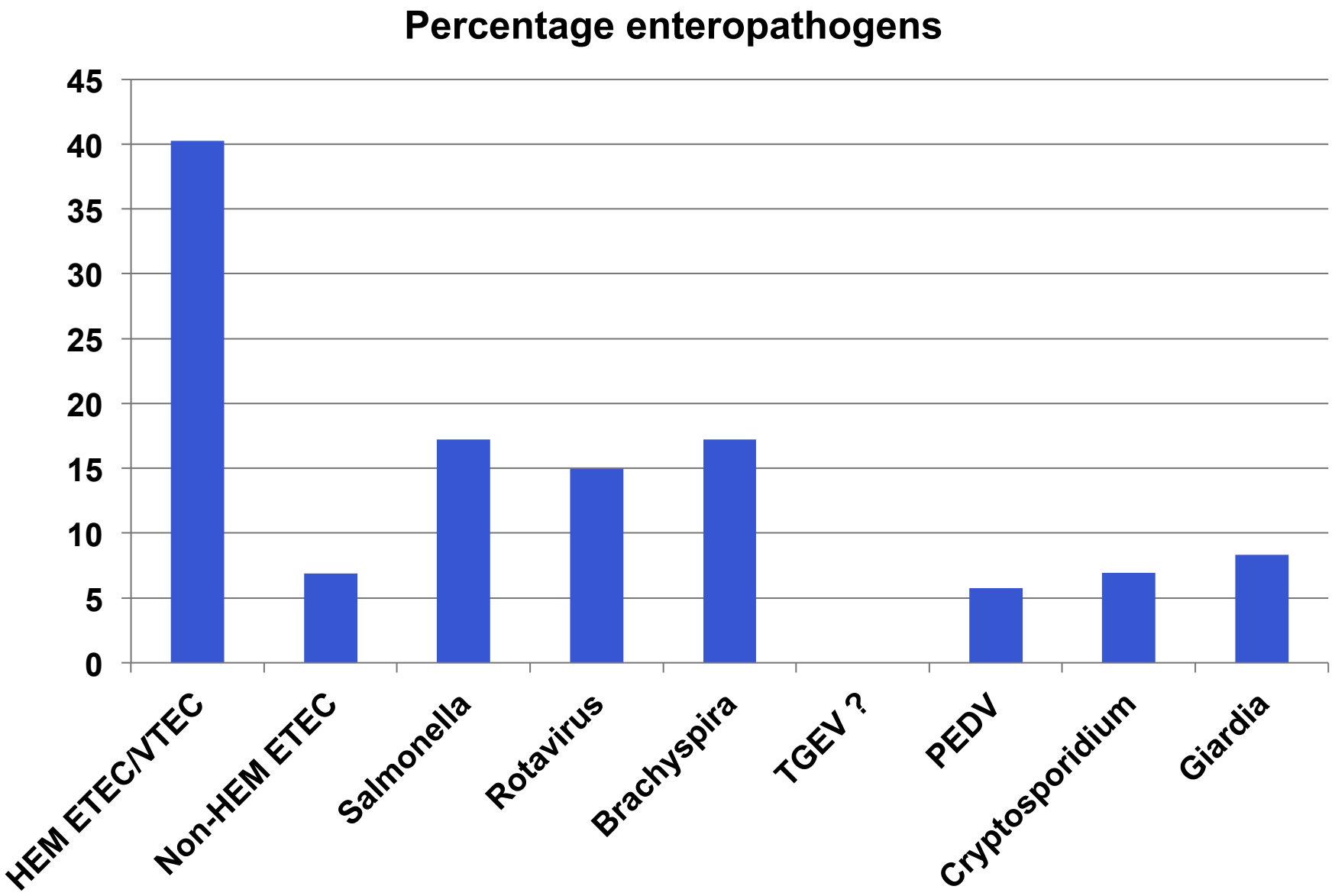
Pigs

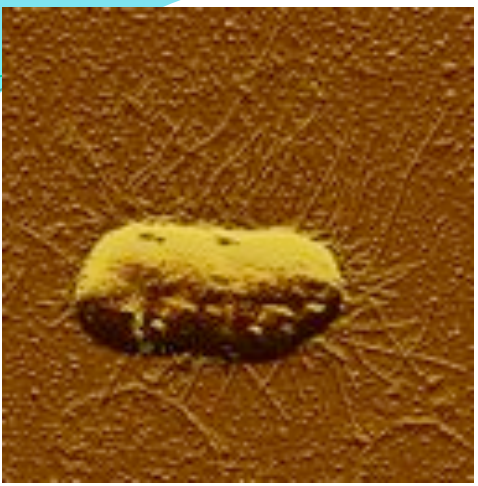
- **Diarrhoea = 11 % of all post-weaning mortality**
- **± 10 million piglets die annually world-wide**
- **50 % is caused by enterotoxigenic *E. coli***
- **The economical losses due to oedema disease are not known**

Bacteria and viruses identified in faeces of pigs post weaning on Belgian farms

Coddens et al., manuscript in preparation



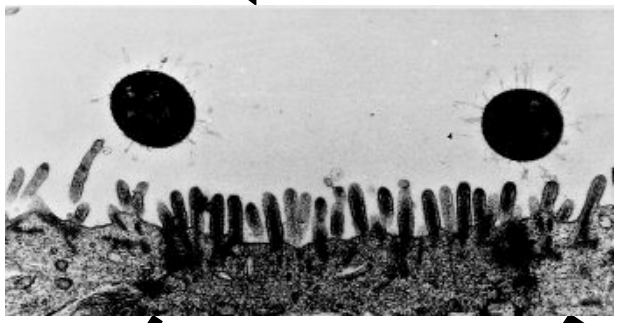
Piglets post-weaning



Enterotoxigenic E. coli (ETEC)
Vérotoxinogenic E. coli (VTEC)

Fimbriae (F4 (K88), F18)

Colonization factors bind to sugars



Enterotoxins (LT, STa, STb, EAST1)

Shiga-toxin (Stx2e)

Post-weaning diarrhoea

Oedema disease

Cox, UGent, 2013

Cox, UGent, 2013

The colonisation factors F4 and F18 and their F18 receptors

F4 and F18 fimbriae differ in structure and in receptor-specificity

Minor subunits

MAJOR subunit FaeG

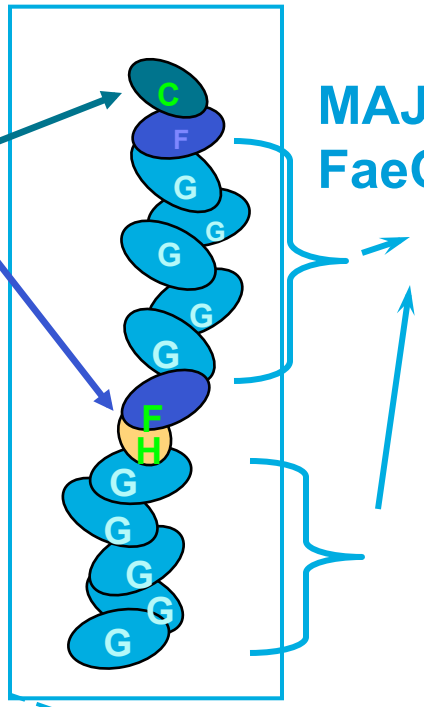
MINOR subunit FedF

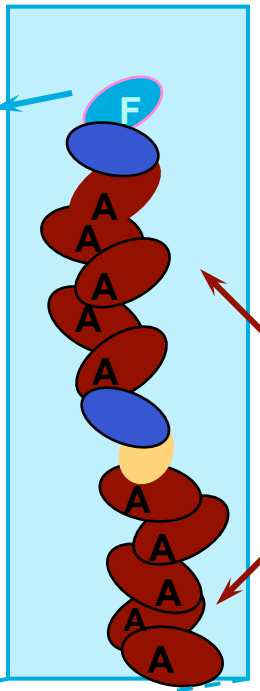
= ADHESIN =

= lectin =

Binds to sugars

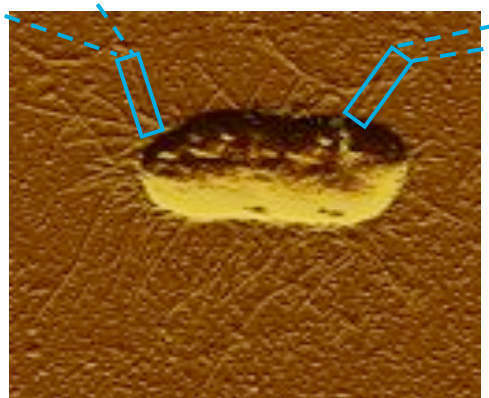
Major subunit





F4 fimbriae

F4ab/ac/ad

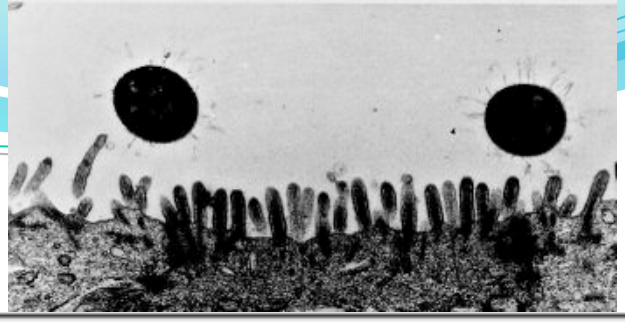


F18 fimbriae

F18ab/ac

Cox, UGent, 2013

F4 receptor phenotype



Phenotype	Adhesiveness	Receptor	Identification of receptor	
			Characterization	Molecular mass (kDa)
A	ab, ac, ad	*bcd*	glycoproteins	45–70
		bc	glycoproteins	210 and 240
B	ab, ac	*bc*	glycoproteins	210 and 240
C	ab, ad	*d*	glycosphingolipid	?
D	ad	*d*	glycosphingolipid	?
E	/	/	**Receptor negative phenotype**	
F	ab	*b*	glycoprotein	74

F4 binding occurs to the sugars and is complex

Van den Broeck et al., 2000

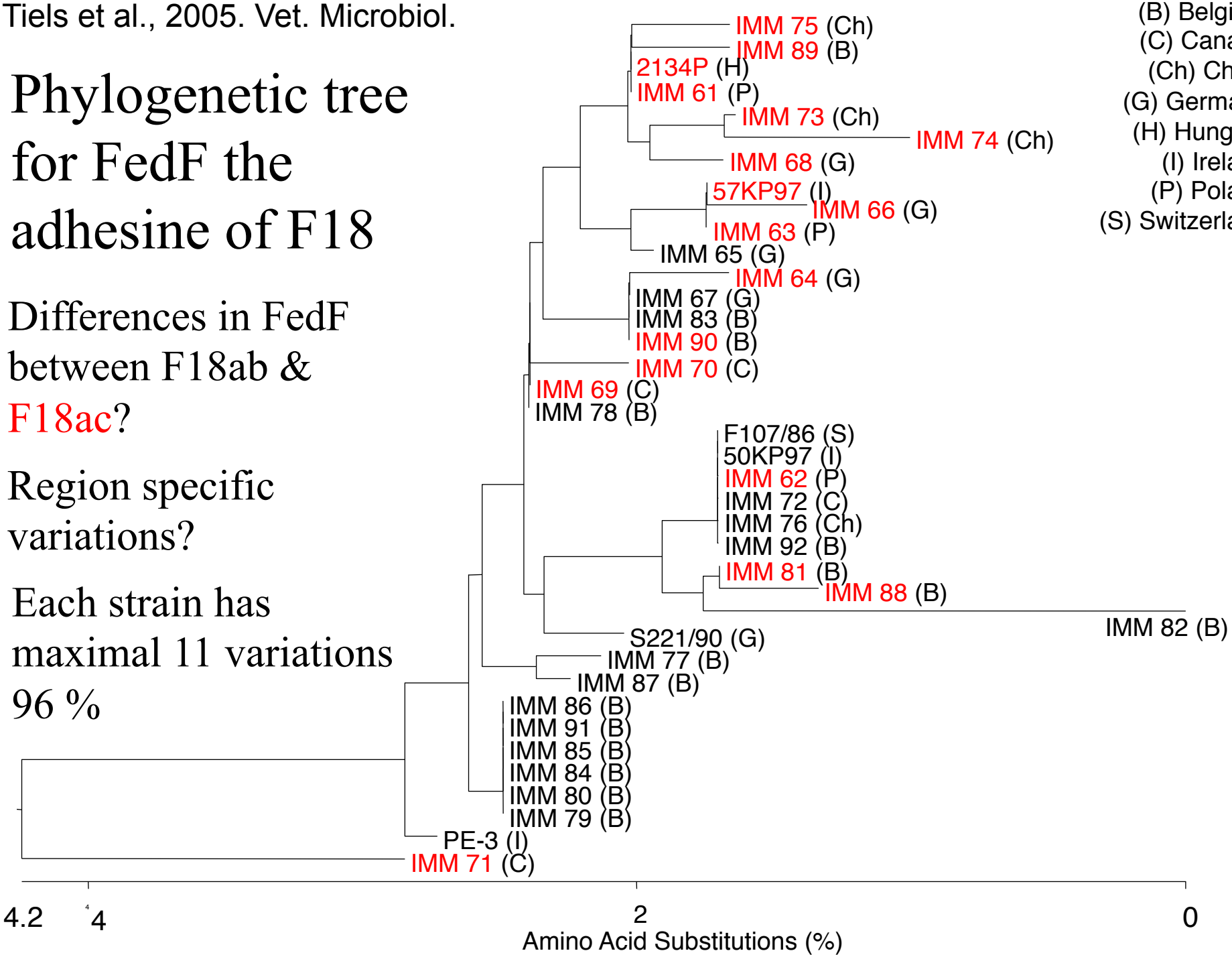
Tiels et al., 2005. Vet. Microbiol.

Phylogenetic tree for FedF the adhesine of F18

Differences in FedF between F18ab & F18ac?

Region specific variations?

Each strain has maximal 11 variations 96 %



Conclusions

F4ab ≠ F4ac ≠ F4ad

FedF 96% identical

No difference between F18ab$^+$ & F18ac$^+$ *E. coli*

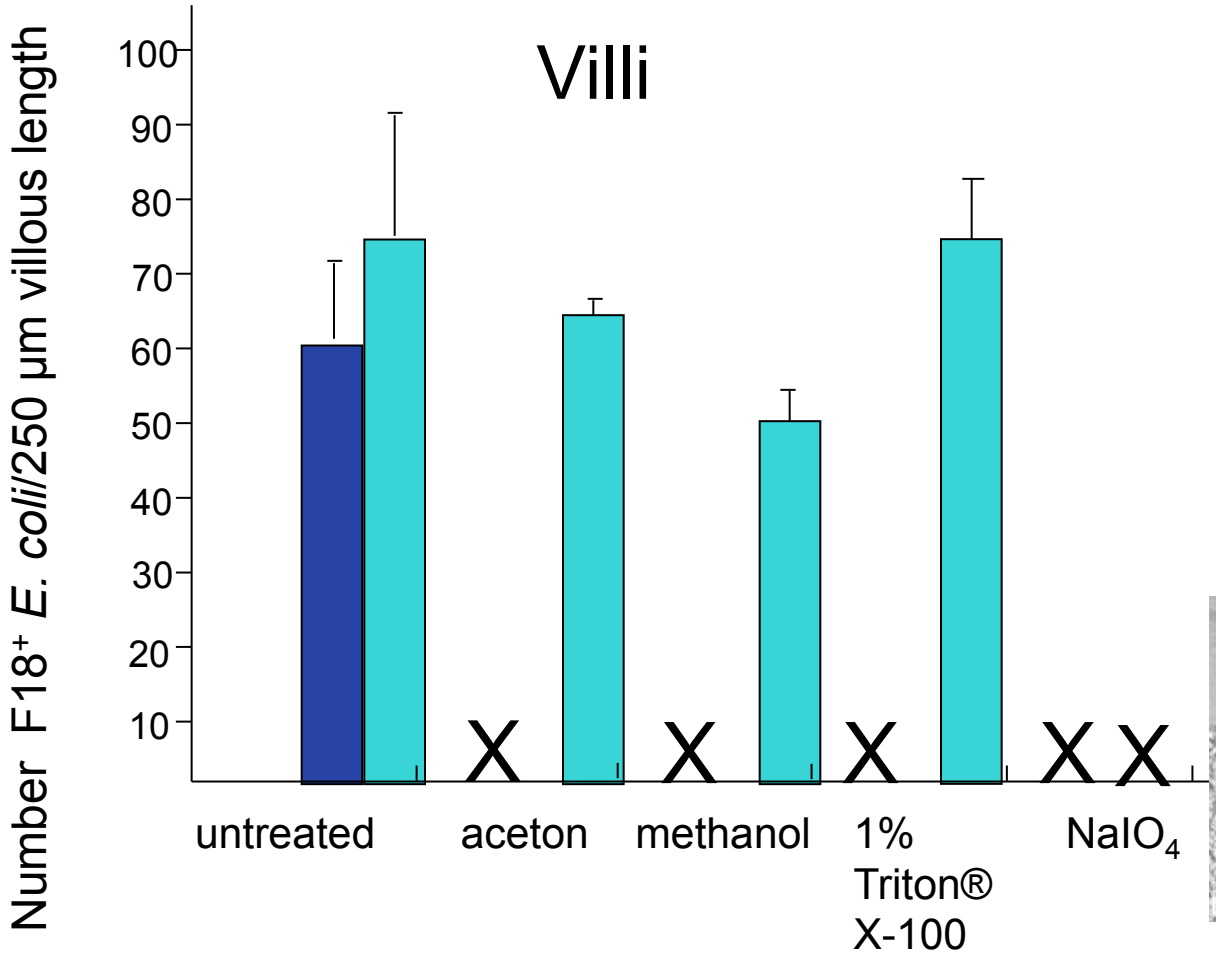
No region specific variation

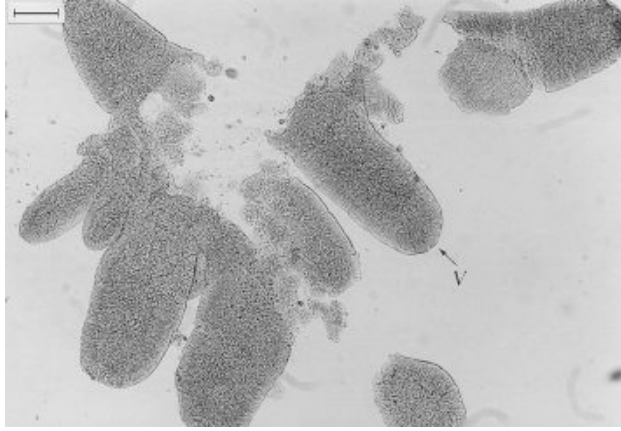
FedF is worldwide conserved

⇓

The same receptors

Wat is the nature of the F18 receptor ?



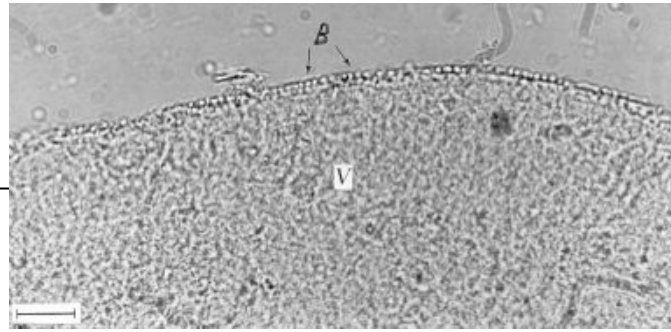


+

F18$^+$ *E. coli* (n = 4)

or

F4ac+ *E. coli* (n = 2)

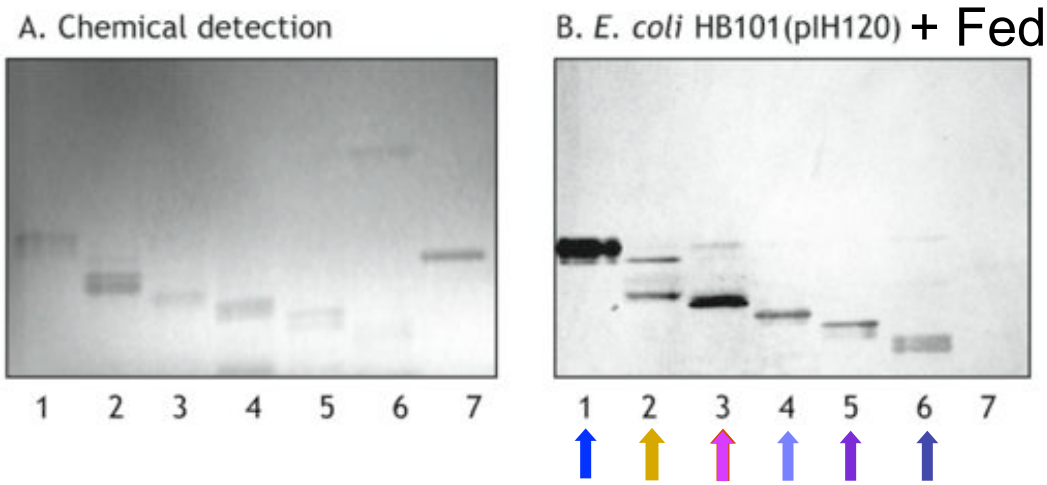


⇒The F18R is a glycolipid

⇒The F4R is a glycoprotein

Coddens et al., 2009. J. Biol. Chem.

Isolation and characterization of F18$^+$ *E. coli*-binding glycospingolipids from blood group O and A pig intestinal epithelium



Non-acid glycosphingolipid fraction

Mass spectrometry

Proton NMR spectroscopy

Blood group 0 => H5 type 1 Fucα2Galβ3GlcNAcβ3Galβ4Glcβ1Cer

Blood group A =>

A6 type 1 GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer

A7 type 4 GalNAcα3(Fucα2)Galβ3GalNAcβ3Galα4Galβ4Glcβ1Cer

A8 type 1 GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4Glcβ1Cer

A9 type 1 GalNAcα3(Fucα2)Galβ3GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer

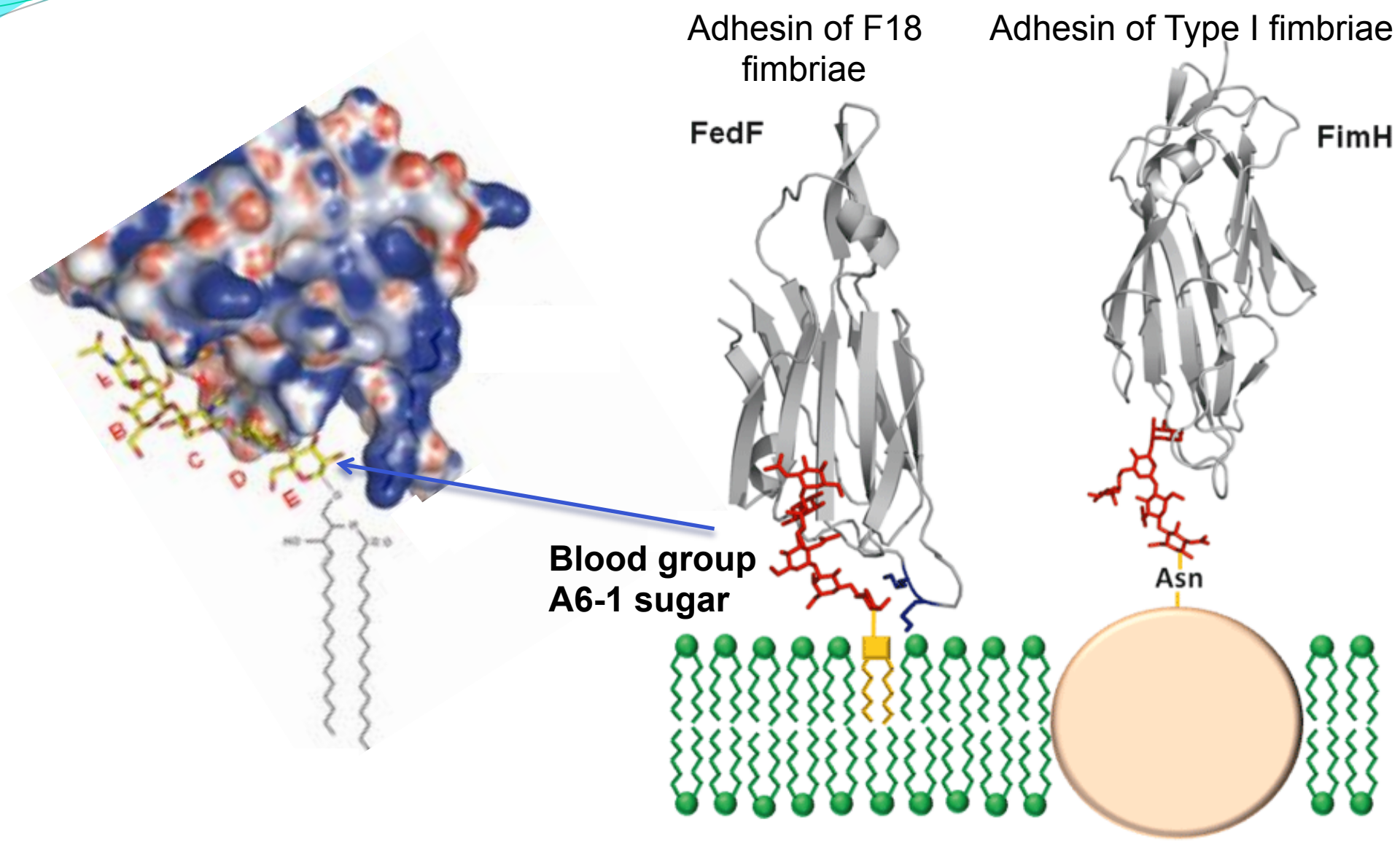
Decaglycosylceramide with terminal HexNAc-(Fuc-)Hex-HexNAc sequence

Undecaglycosylceramide with terminal HexNAc-(Fuc-)Hex-HexNAc sequence

Dodecaglycosylceramide with terminal HexNAc-(Fuc-)Hex-HexNAc sequence

Coddens et al., 2009. J. Biol. Chem.

Structural insight in histo-blood group binding by the F18 fimbrial adhesin FedF



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

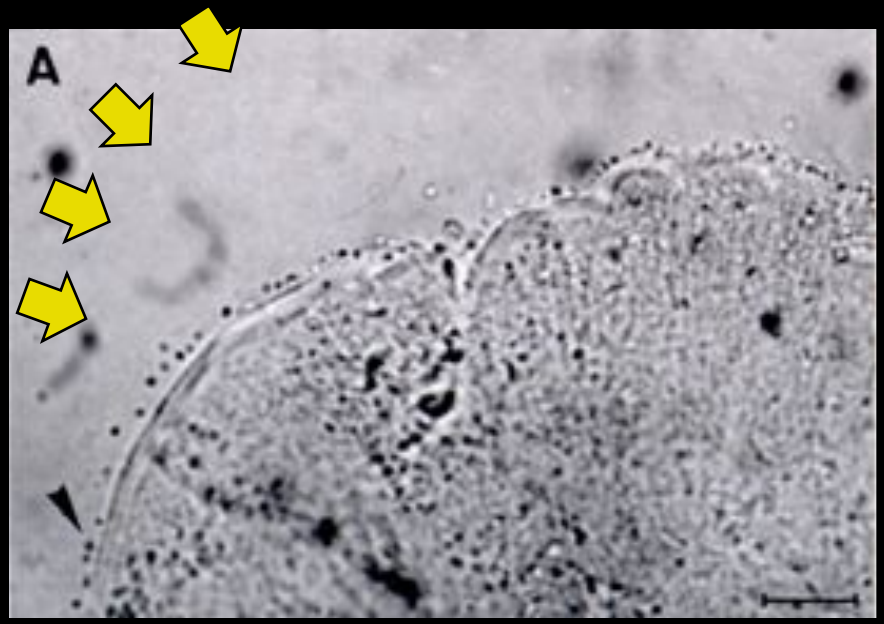
Conclusion

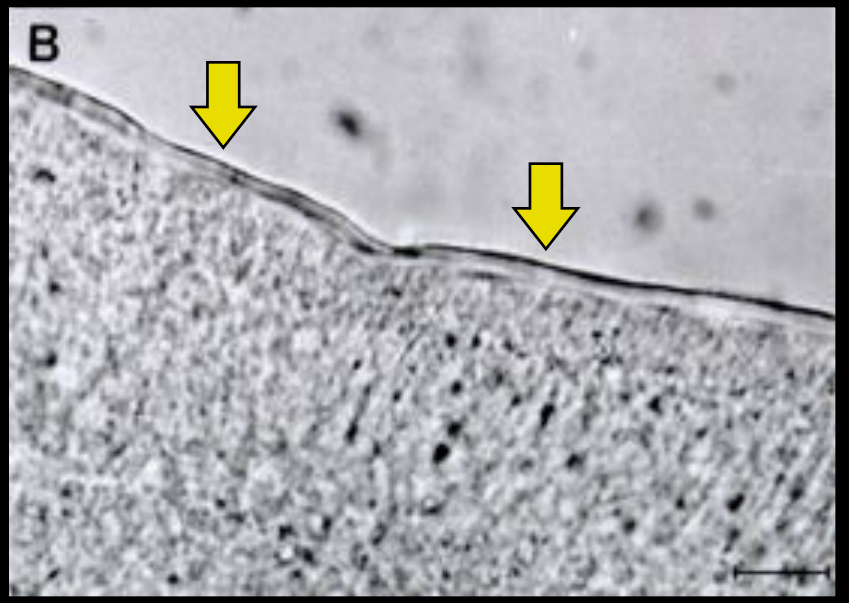
F18 adheres to blood group A (A6, A8, A9 type 1, A7 type 4) **and O** (H5 type 1) **sugars**

The expression of fimbrial receptors as determined by *in vitro* adhesion

F4R +

F4R- (Natural knock-out)

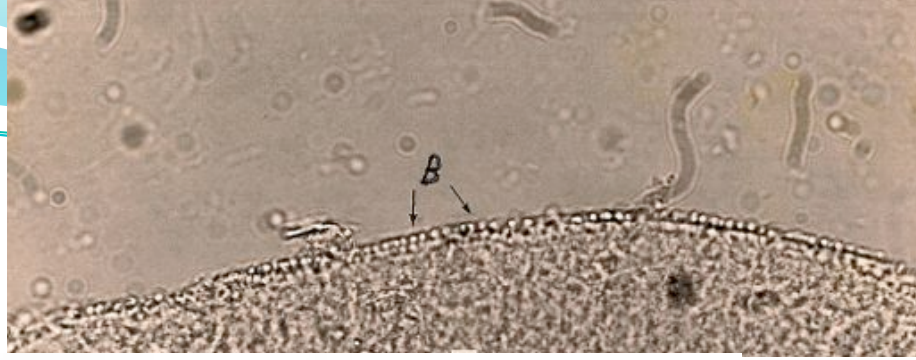


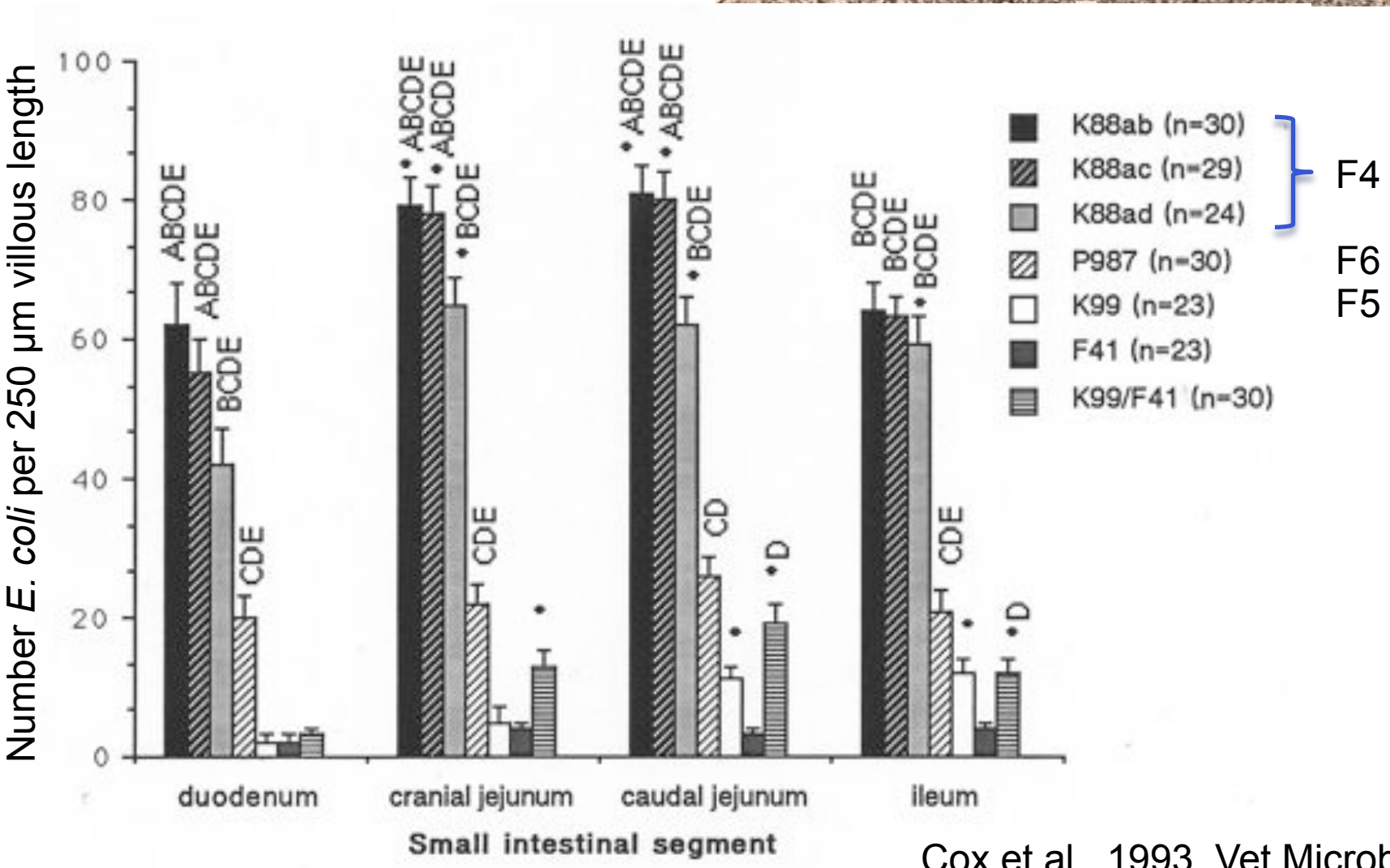


bar: 10 µm

Van Den Broeck et al., 1999. Inf Imm

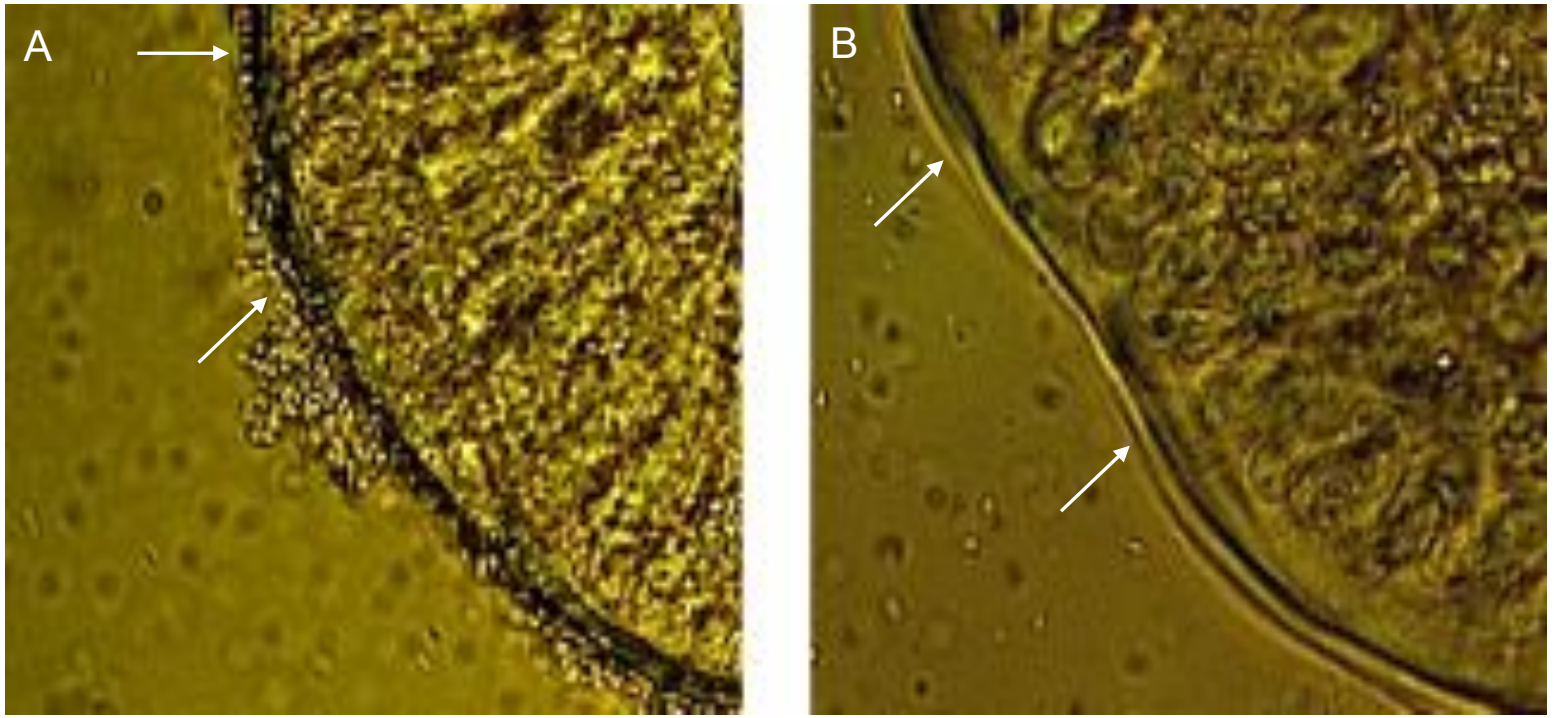
Binding of ETEC to villi of 4- to 5-week-old piglets





Cox et al., 1993. Vet Microbiol

F18 receptors



***In vitro* villous adhesion assay**

F18^{+} *E. coli* adhesion ⇩ F18R positive piglets

No adhesion ⇩ F18R negative piglets

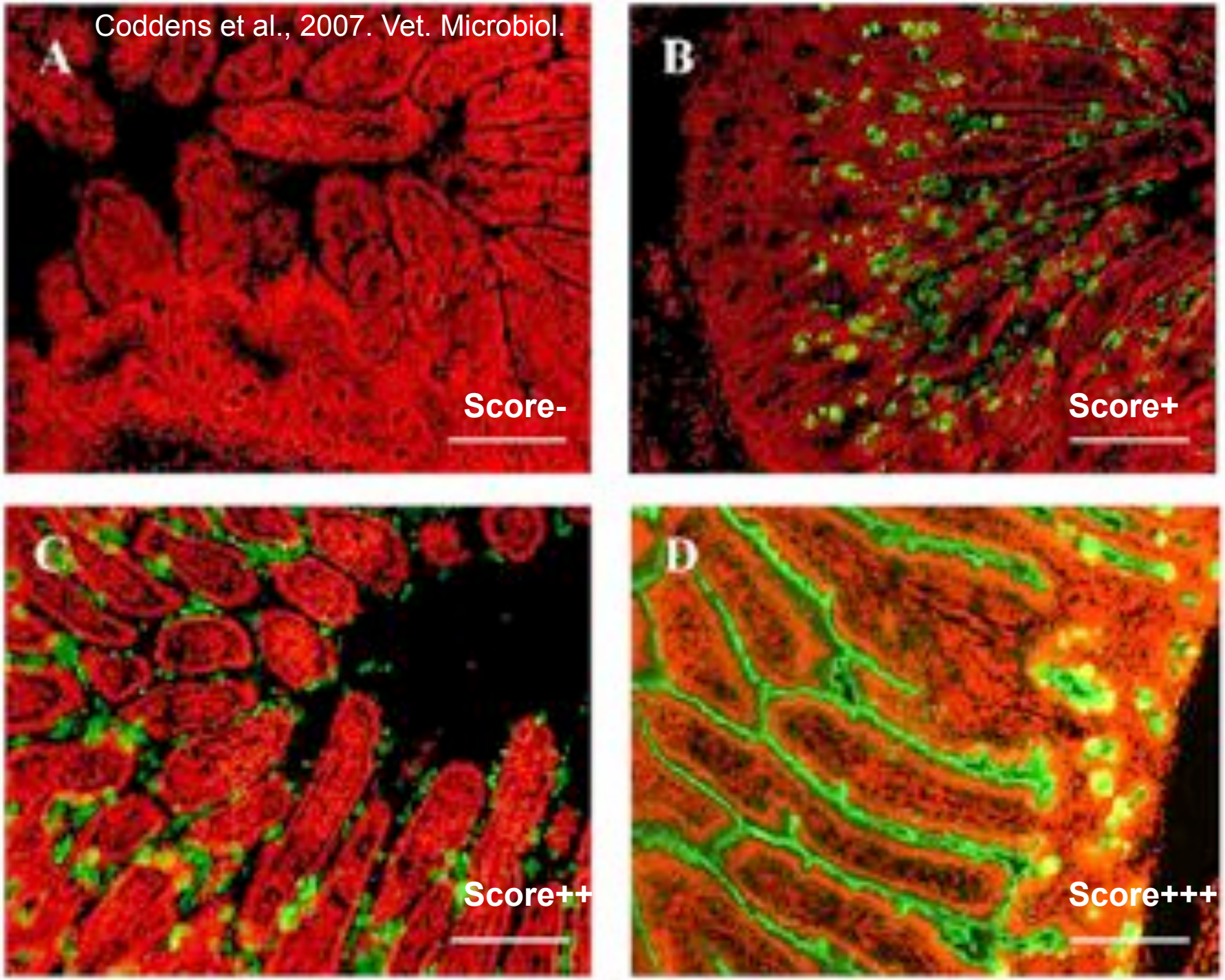
Expression of F18R is function of age

74 porcelets were tested

Number of piglets	Age (weeks)	FUT Expression			Number of piglets with F18+*E. coli* adhering to 250 µm villous length		
		G/G Susceptible	G/A	A/A Res.	<5 bacteria -	5-30 bact +	> 30 bact ++
4	0	1	2	1	4		
4	1,5	3	1		3	1	
12	3	4	8		5	5	2
5	4	2	2	1	3		2
8	5 tot 6	8				6	2
5	8	3	2		2	2	1
6	9	3	2	1		6	
5	10 tot 11	1	2	2	2	1	2
5	12	3	2			3	2
8	13	6	2		3	5	
5	14	3	1	1		3	2
4	17 tot 18	2	2				4
3	22 tot 23	2	1		1		2

G/G or G/A = susceptible A/A = resistent

Coddens et al., 2007. Vet. Microbiol.

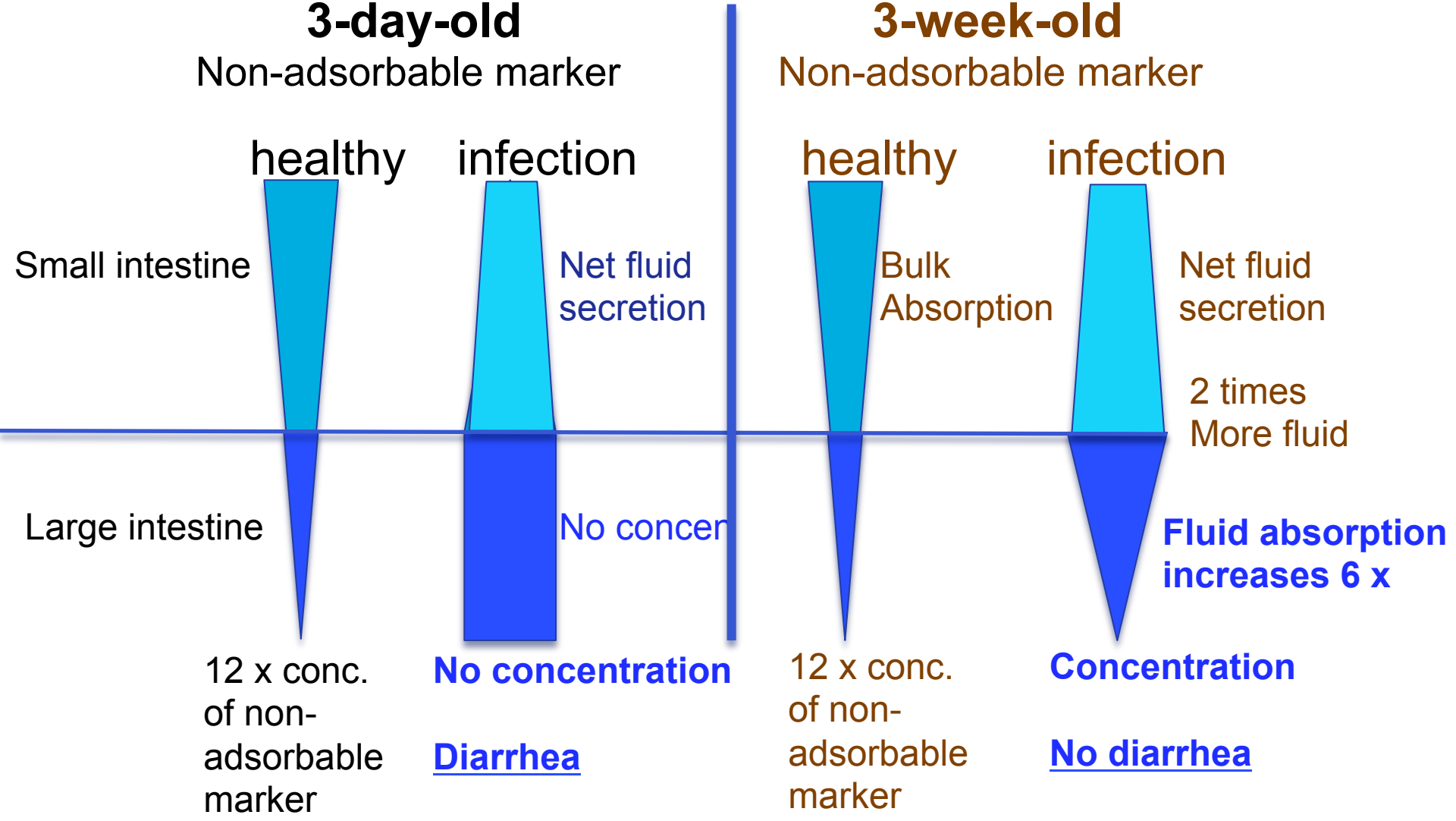

Coddens et al., 2007. Vet. Microbiol.
A
Score-
B
Score+
C
Score++
D
Score+++

Conclusion

- Expression of the F4R is irrespective of age
- Expression of the F18R is age-dependent
- There is sufficient F18R expression from 3 weeks of age in F18R positive pigs

Enterotoxins induce diarrhoea

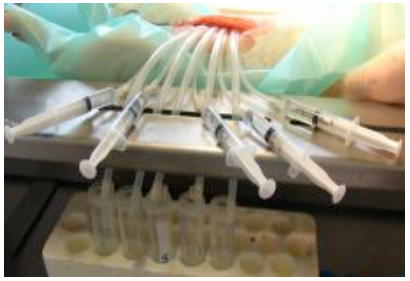
The weaned pig's large intestine has a higher capacity to absorb fluid

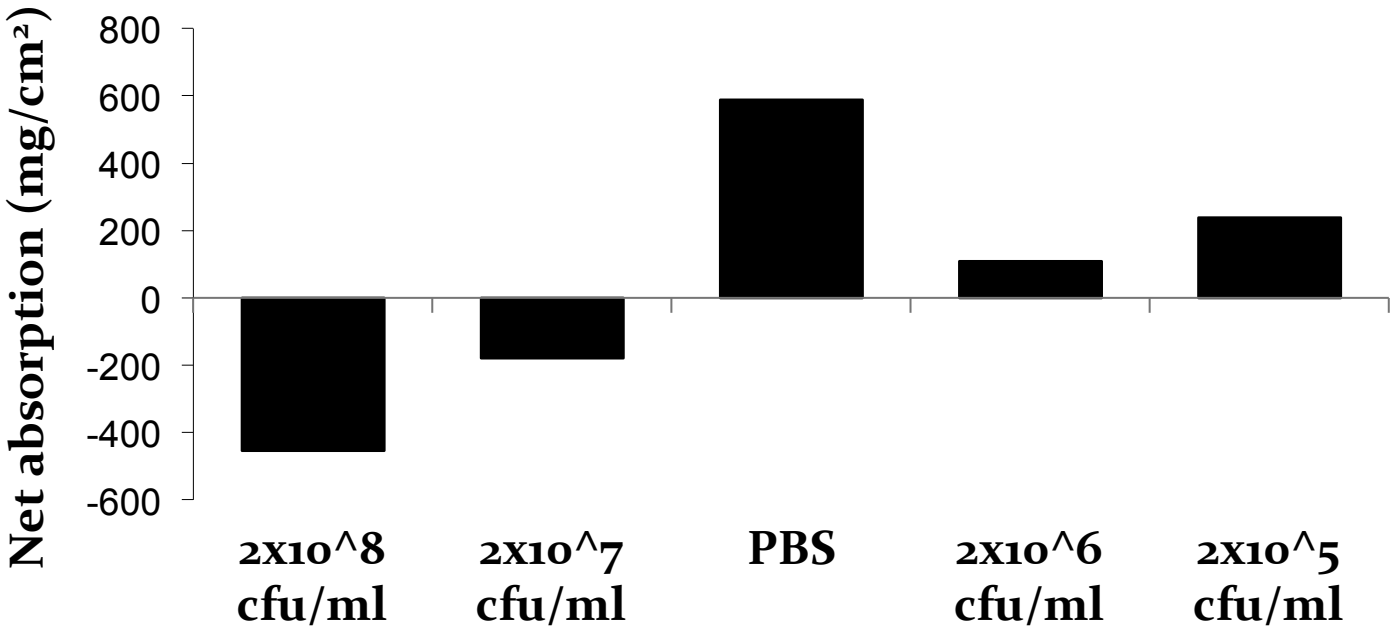


Adapted from Argenzio et al, 1984. Gastroenterology

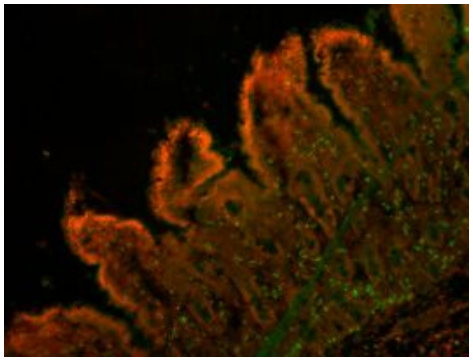
SMALL INTESTINAL SEGMENT PERFUSION (SISP)

F4R+ pig
8h perfusion

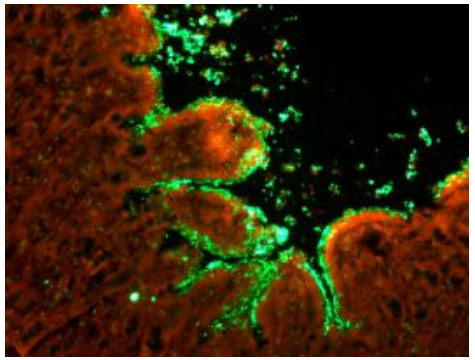




Adhesion of the F4+bacteria:



PBS

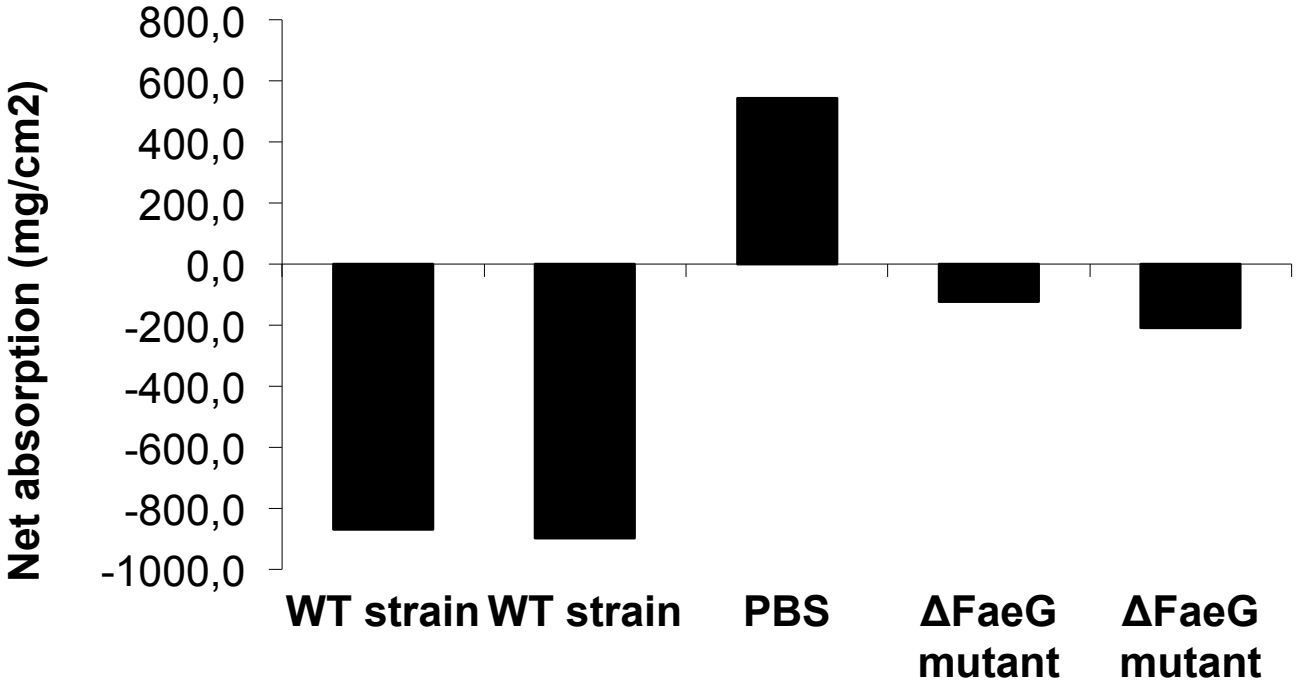


ETEC GIS26

Loos and Cox, UGent, 2013, unpublished results

IMPORTANCE OF F4 MEDIATED ADHESION

- F4R$^+$ pig, **8h** perfusion
- Compare wild type with mutant strain lacking F4 (deletion of FaeG subunit)



F4 mediated adhesion not necessary but stronger effect

Loos and Cox, UGent, 2013, unpublished results

Deletion mutants: phenotypes

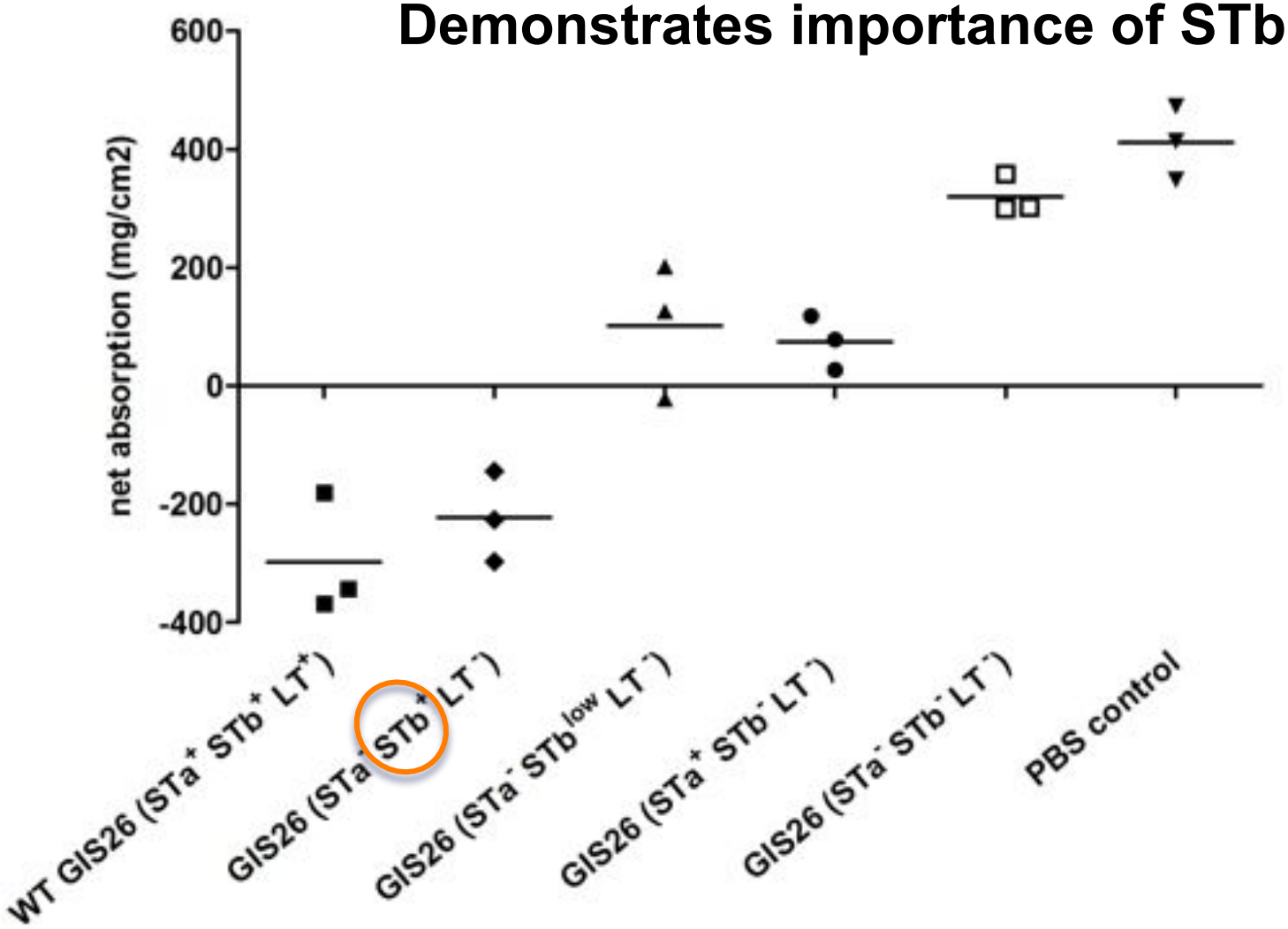
- Quantitative detection of enterotoxin expression *in vitro*
- Detection limits in ng range
- Some mismatches with genotype: gene regulation?

Genotype	Phenotype			Strain designation
	STa	STb	LT	
wild type	+	+	+	GIS26(STa^{+} STb^{+} LT^{+})
ΔeltAB	-	+	-	GIS26(STa^{-} STb^{+} LT^{-})
ΔestBΔeltAB	+	-	-	GIS26(STa^{+} STb^{-} LT^{-})
ΔestA	-	Low	-	GIS26(STa^{-} STb^{low} LT^{-})
ΔestAΔestB:KAN	-	-	-	GIS26(STa^{-} STb^{-} LT^{-})

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

Small intestinal segments were infected and subsequently perfused during 4 hours Demonstrates importance of STb

N = 3 F4R+



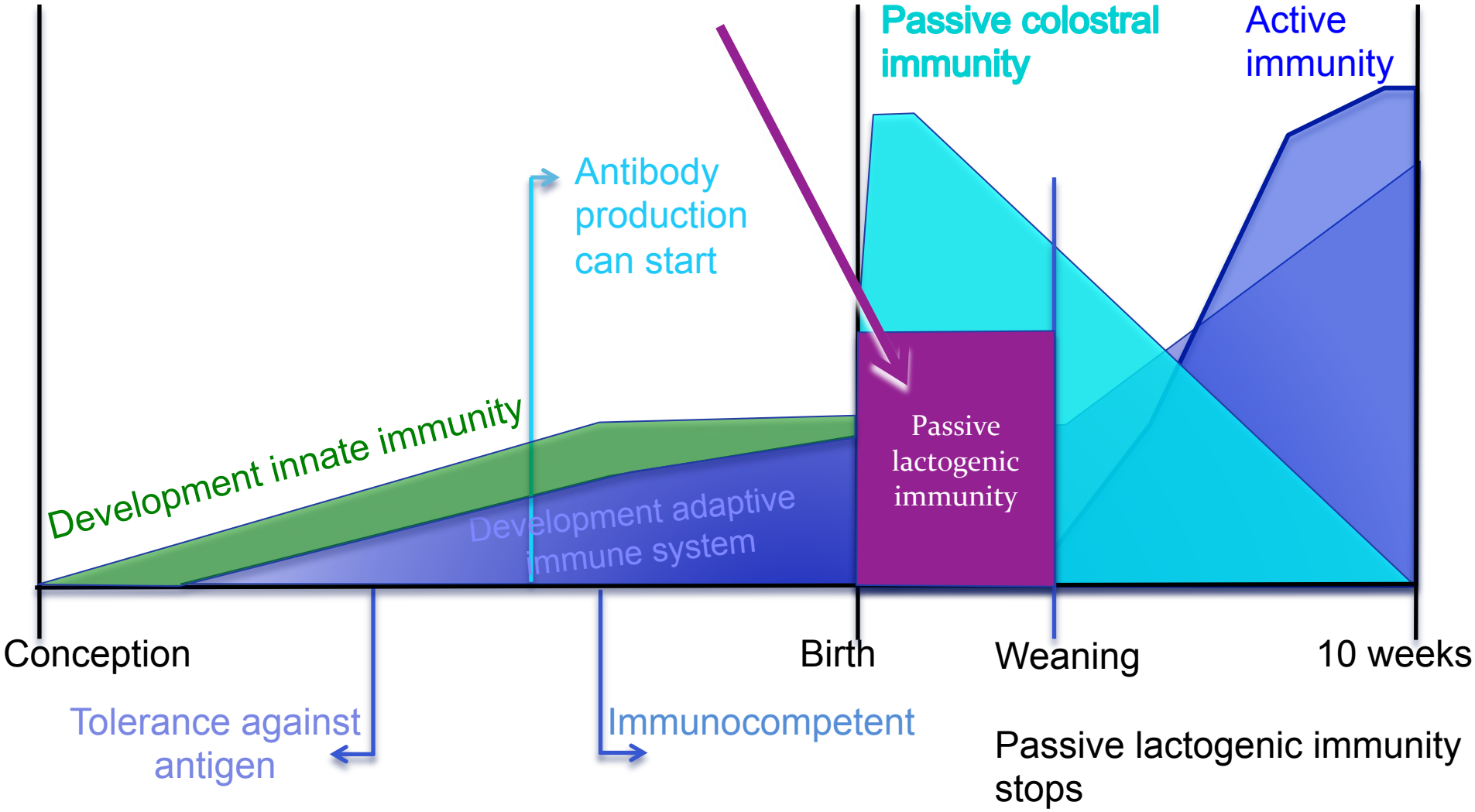
Loos et al., 2012. PLoS ONE 7(7): e41041

The weaning period

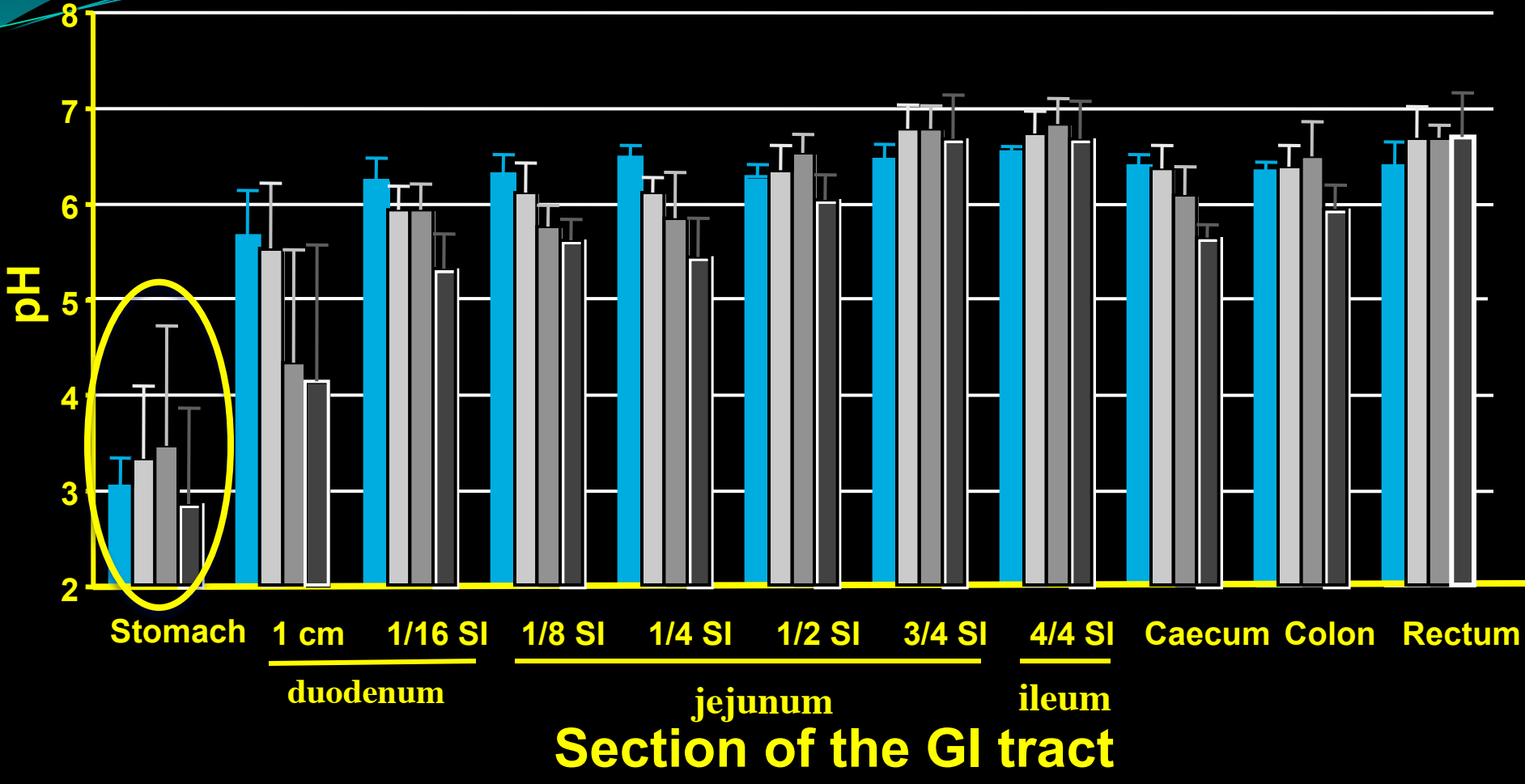
Passive Immunity and mucosal immune response

- **Neutralize enteropathogens**
- **Neutralize oral vaccines/antigens**
- **Prevent intestinal immune response**

Cox, UGent, 2013

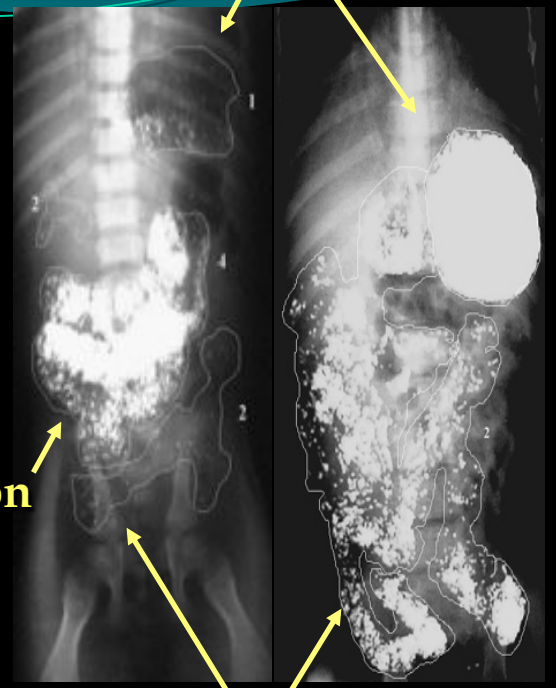


pH of gastrointestinal contents



Snoeck et al., 2004. Vet. Microbiol, 94, 143– 153

Gastric emptying



Gastric emptying for liquids = fast = in suckling piglets 100% within 2 hours

Snoeck et al., 2004. Journal of Controlled Release 94, 143– 153

Changes in Gastrointestinal Physiology

- Proximo-distal gradient reduction in **villus length** along the small intestine
- Decreased activity of most **pancreatic enzymes**
- Decreased activities of **brush border enzymes**
- Increased **para-cellular permeability** in proximal jejunum
- **Trans-cellular permeability** decreased in proximal jejunum and increased in the mid-jejunum
- Activation of **mucosal mast cells**, **enteric nerves**, prostanoid pathways
- A broad spectrum of **adaptive immune variables suppressed** the first week after weaning
- Several variables of the **innate immune system seem to be stimulated** immediately after weaning
- Transient **over-expression of inflammatory cytokines** (IL-1, IL-6, TNF-alfa) along the intestine

J.-P. Lallès, Archiva Zootechnica 11:1, 5-15, 2008
Juul-Madsen et al., Vet Imm Immunopathol, 2010

Early and late Immune response

Cox, UGent, 2013

FOLLICLE-ASSOCIATED EPITHELIUM

Pathogens
Particulated antigens

M cell

Danger

Epithelium

DC

IgA production

Mucosa-associated lymphoid tissue

Immunity

T effector cels

MUCOSA

Non-replicating soluble antigens

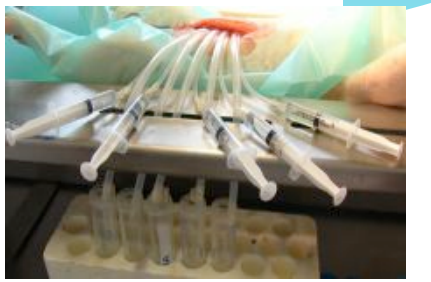
Dentritic cells

Immature antigen - presenting cels

Draining lymph node

Oral tolerance

THE EARLY RESPONSE MICRO-ARRAY ANALYSIS



- 3 F4R$^+$ pigs different gut segments infected with
  - **Uninfected control versus wild type** strain infected segment = what is the general ETEC respons:
  - **Mutant strain versus wild type** strain infected segment = What is the role of enterotoxins:
- mRNA from intestinal segments after 4h perfusion
- Porcine Genome Array (Affymetrix 23,937 probe sets ➔ 20,201 *Sus scrofa* genes)

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

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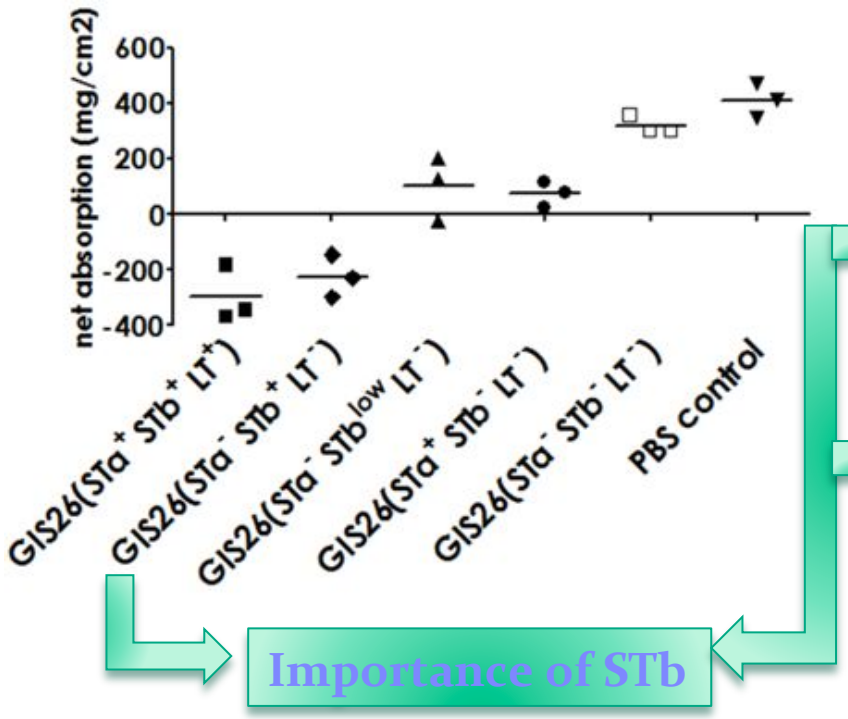
Functional response



Gene expression

13 transcripts up-regulated by GIS26 wild type

Gene	Gene symbol	Probe Set ID	Log2 ratio
Matrix metalloproteinase 3	*MMP3*	Ssc.15927.1.S1_at	4.18
		Ssc.15927.2.S1_at	4.16
		Ssc.15927.2.A1_at	2.90
Interleukin-17A	*IL17A*	SscAffx.23.1.S1_at	3.68
Pancreatitis associated protein	*PAP (REG3A)*	Ssc.16470.1.S1_a_at	3.10
Interleukin-1 beta	*IL1B*	Ssc.17573.1.S1_at	2.72
		Ssc.15601.1.A1_s_at	2.65
Interleukin-1 alpha	*IL1A*	Ssc.113.1.S2_at	2.68
		Ssc.113.1.S1_at	2.30
Dual oxidase 2	*DUOX2*	Ssc.33.1.S1_at	2.33
Matrix metalloproteinase 1	*MMP1*	Ssc.16013.1.S1_at	2.05
Ectoderm-neural cortex protein 1	*ENC1*	Ssc.30857.1.S1_at	2.05
interleukin-1 receptor antagonist	*IL1RN*	Ssc.16250.1.S2_at	2.01

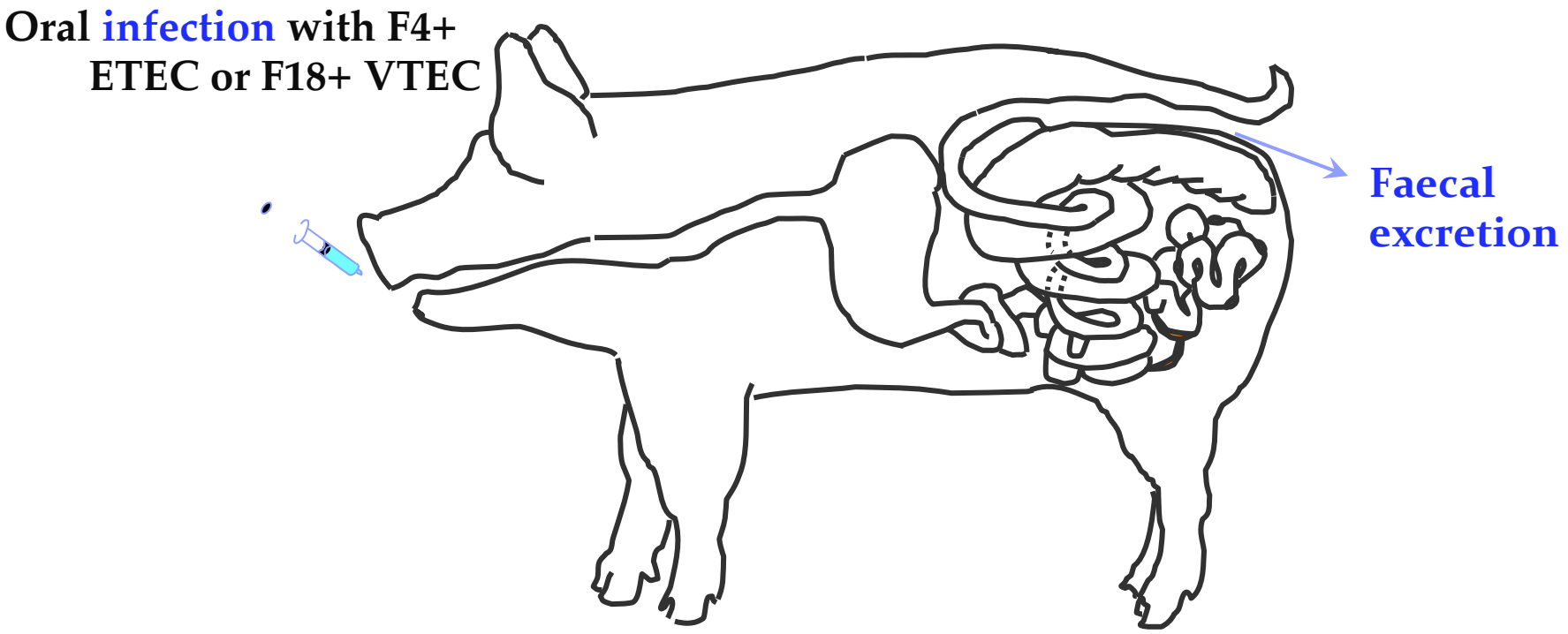
Conclusions:

ETEC induces

1) non-toxin related general antibacterial response (PAP,MMP1, IL8,…)
2) Important role for STb in small intestinal secretion as well as in the ETEC induced immune response (MMP3, IL17, IL1)

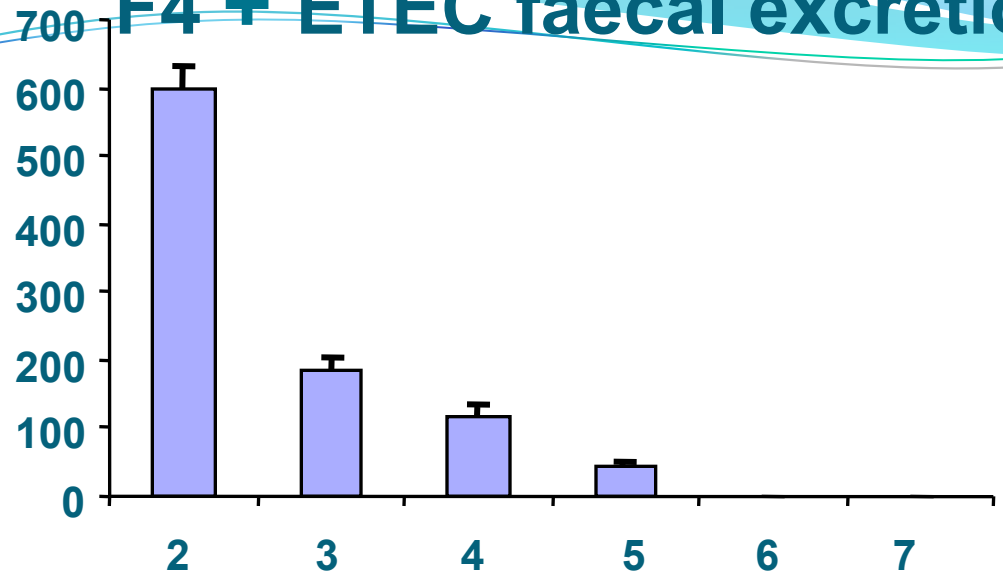
Loos et al., 2012. PLoS ONE 7(7): e41041

Adaptive Immune response following infection with F4+ ETEC or F18+ VTEC



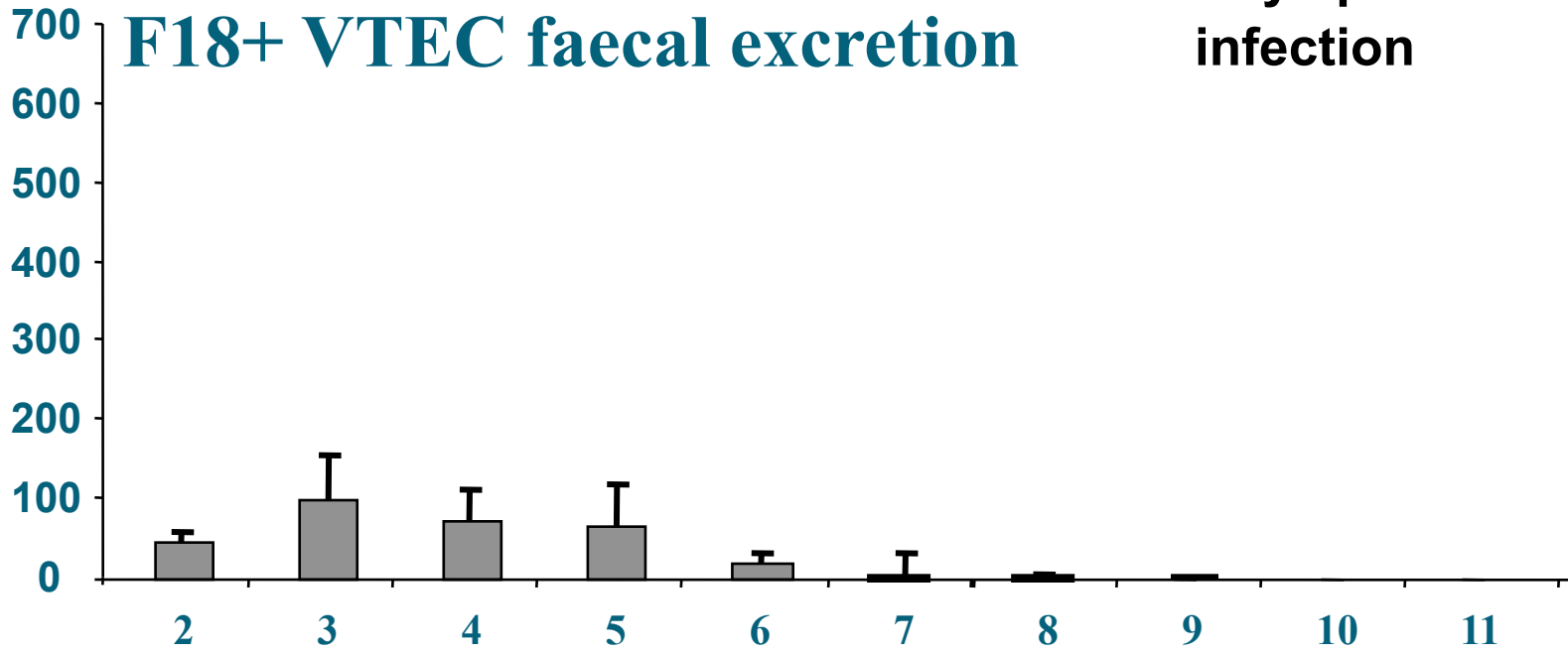
Verdonck et al., 1999. Vaccine

F4 + ETEC faecal excretion



10^6 *E. coli* per g faeces

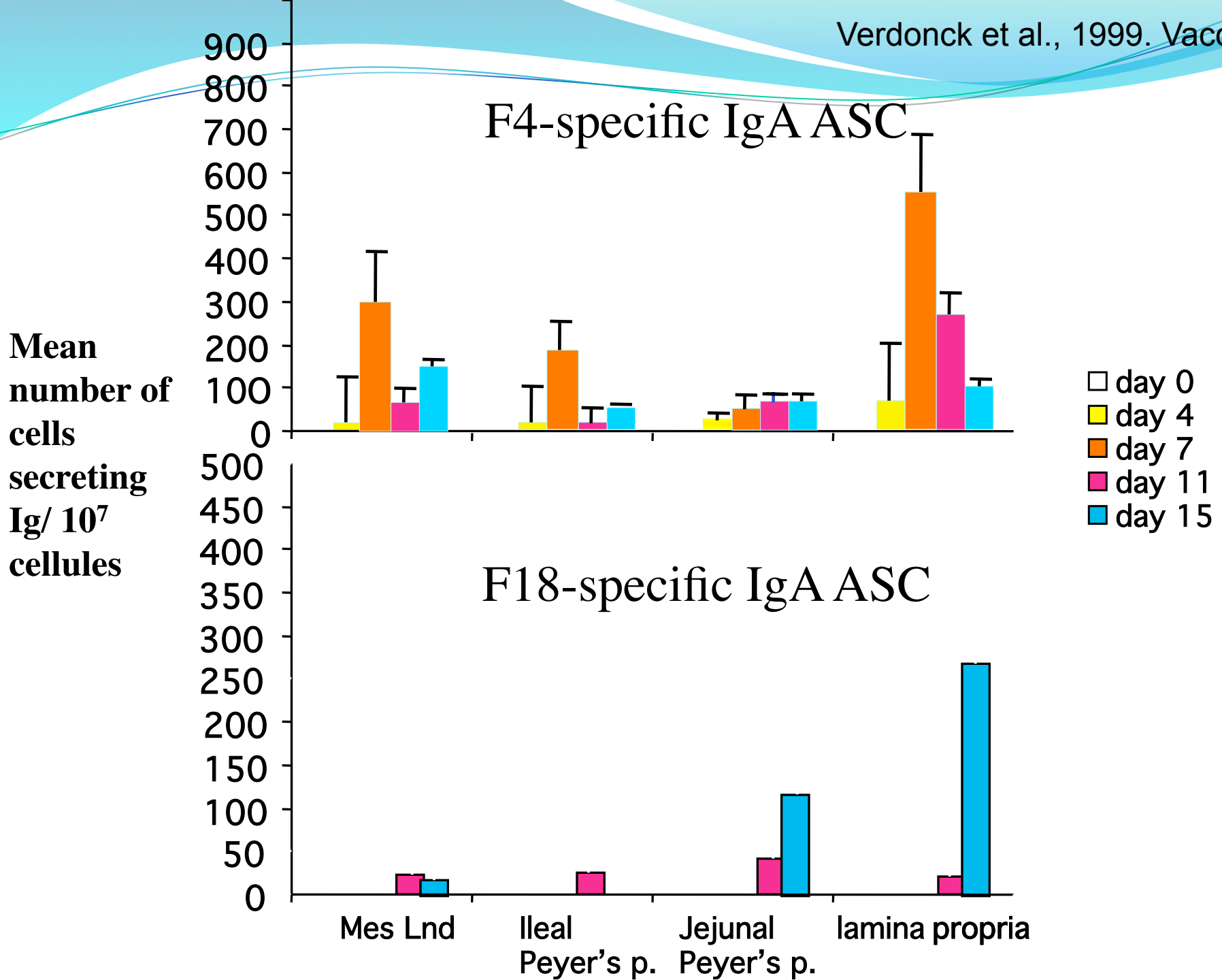
F18+ VTEC faecal excretion



Days post infection

Verdonck et al., 1999. Vaccine

Verdonck et al., 1999. Vaccine



Conclusions

Infection with F4+ ⟺ F18+ *E. coli*

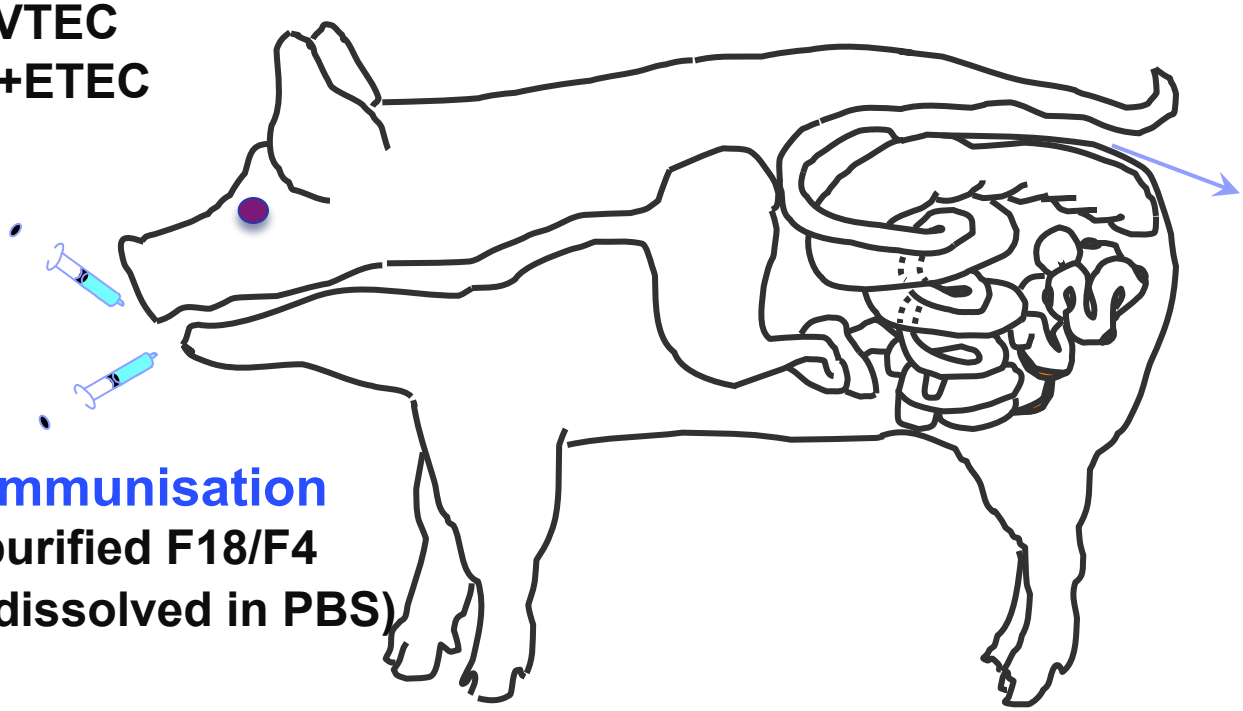
- Colonization difference
  - Rapid (1st week) ⟺ Slower (2nd week)
  - High ⟺ Lower excretion
- Difference in antibody response
  - Quick ⟺ Slow

Different fimbriae (affinity)
Different toxins (enteroxins => secretion; STb => inflammatory, LT => adjuvants)

Verdonck et al., 1999. Vaccine

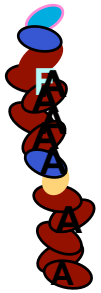
Immunization with F4 or F18 fimbriae via the oral route

2. **Infection challenge** with
F18+ VTEC
or F4+ETEC



3. **Faecal excretion of** F18+ VTEC/ F4+ ETEC

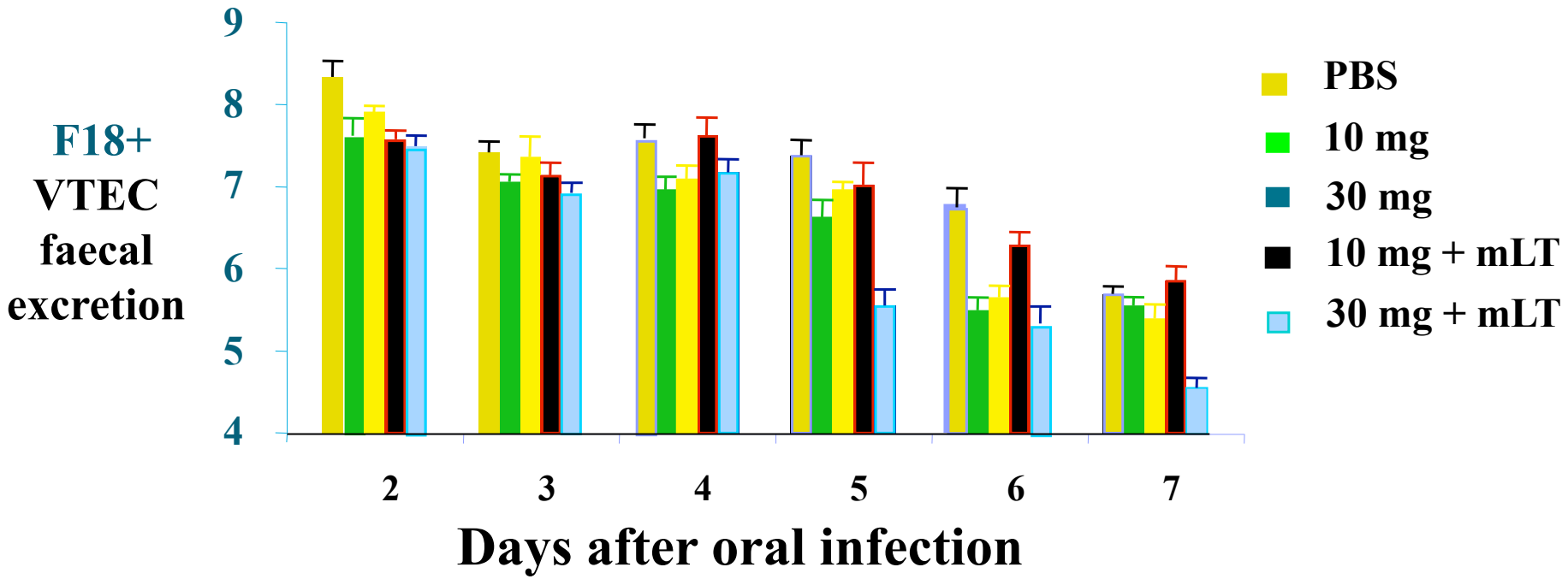
1. **Oral Immunisation** with purified F18/F4 (and dissolved in PBS)



Van den Broeck et al., 1999. Infect Imm

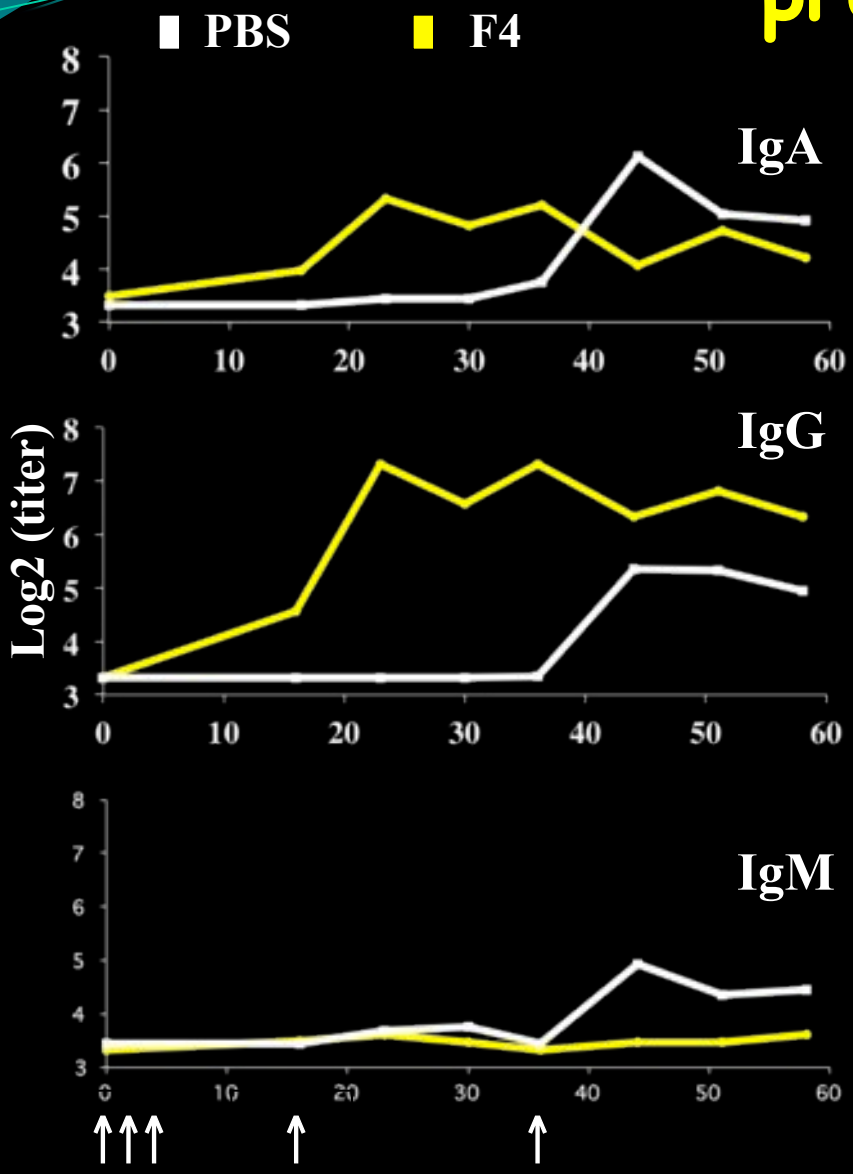
Verdonck et al., Vet Imm Immunopath. 2007

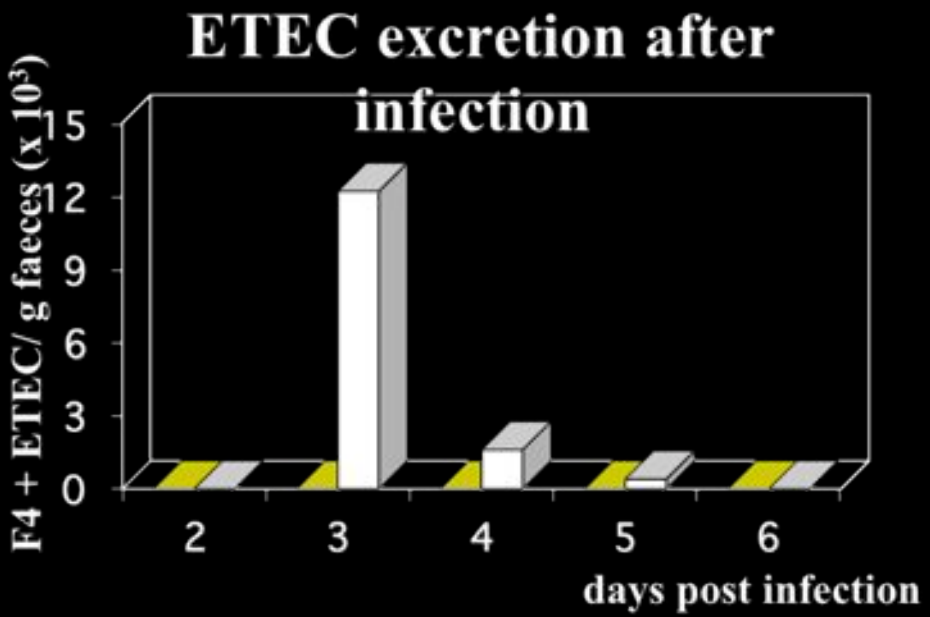
Oral vaccination with F18 fimbriae does not protect



Verdonck et al., Vet Imm Immunopath. 2007

The mucosal response against F4 is protective





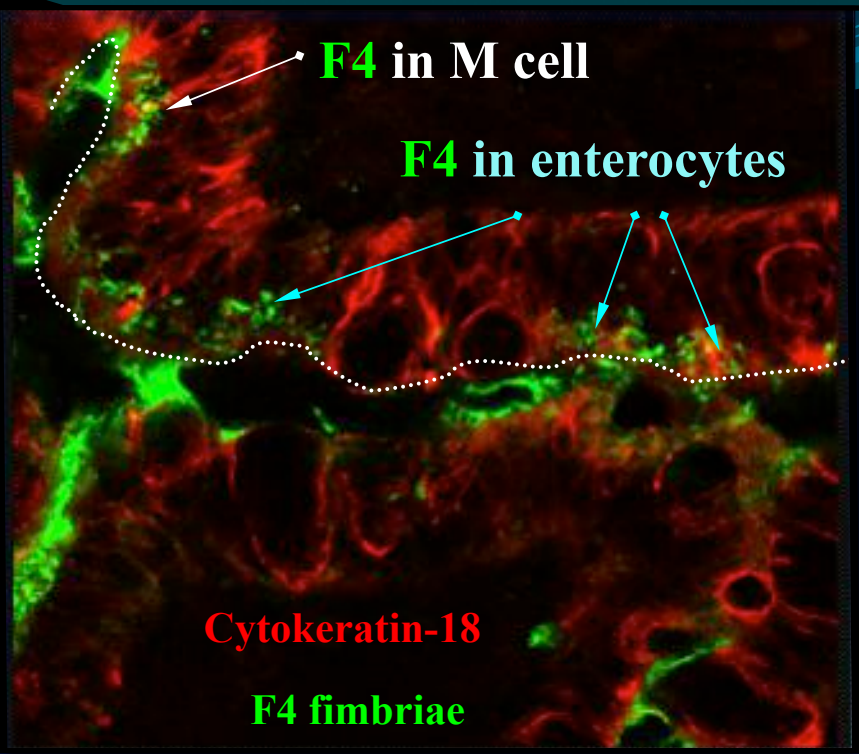
=> Oral F4 induces protective mucosal response !

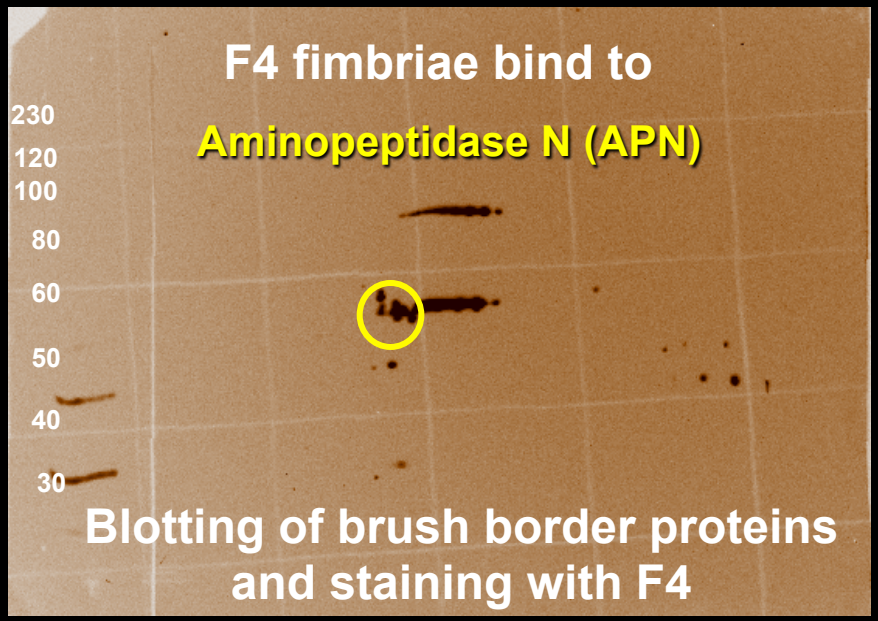
Van den Broeck et al., 1999. Infect Imm

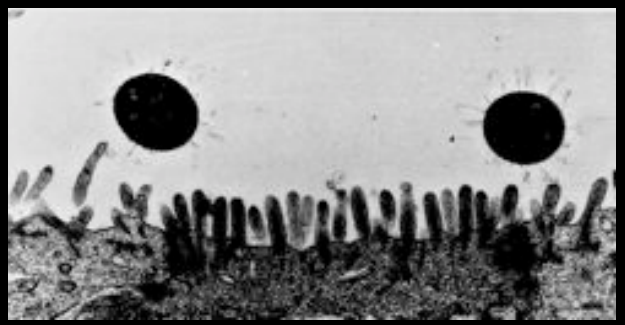
Binding and uptake of F4 fimbriae

Ligated loops injected with F4

Snoeck et al., 2008. Vet Imm Immunopath.







Melkebeek et al., 2012. Mucosal Immunology

Summary

- Weaning = opportunity for ETEC/VTEC to colonize the intestine
  - Decreased food intake
  - Decreased gastrointestinal transit
  - Decreased digestion
  - Absence of passive protection
  - Suppression of adaptive immunity
- Virulence factors influences colonization and immunity
  - Different fimbriae => different affinity => slower or faster adhesion
  - Different fimbriae differ in immunogenicity
  - Enterotoxins induce fluid secretion influencing colonization
  - Enterotoxins can influence the immune response => inflammation, adjuvanticity
- Immunity should neutralize toxins and/or at least prevent colonization in the very early phase

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