UNIVERSITEIT GENT

Faculty of Veterinary Medicine

PROVAXS

Post-weaning *E. coli* infections in pigs and importance of the immune system

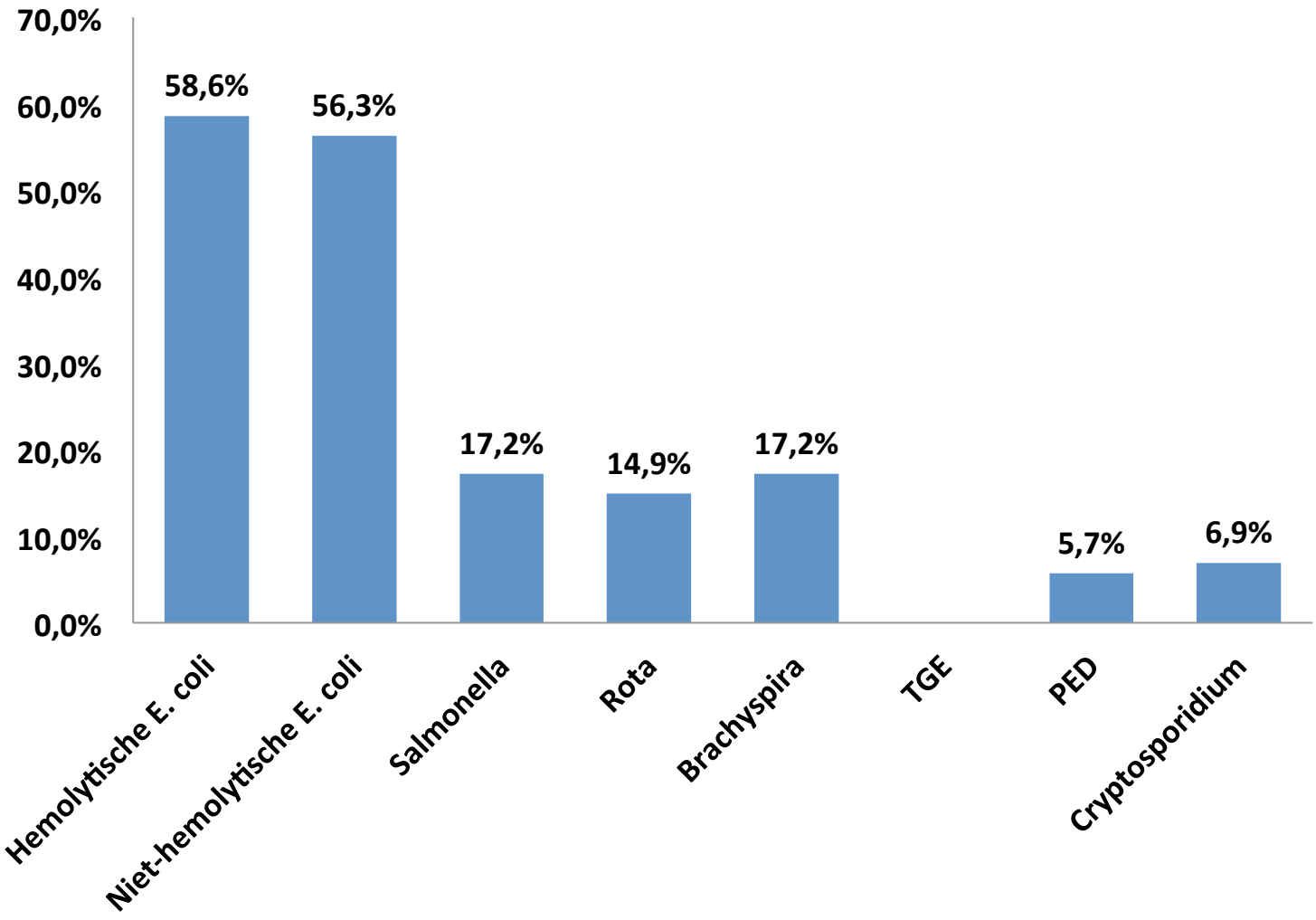
Eric Cox, Laboratory of Immunology, Fac. Vet. Med., UGent, Belgium

Eric.Cox@Ugent.be

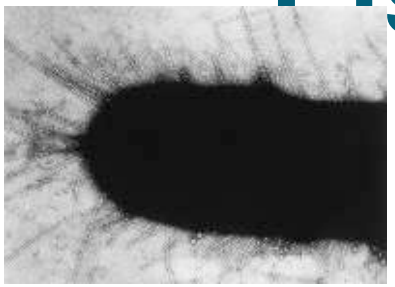
Pigs

- **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 postweaning on Belgian farms

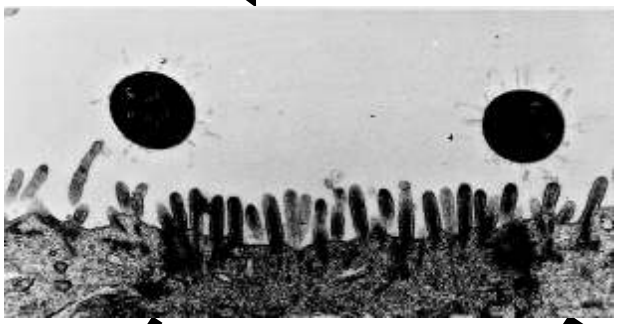


Piglets post-weaning



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

Fimbriae (F4 (K88), F18) **colonisation**



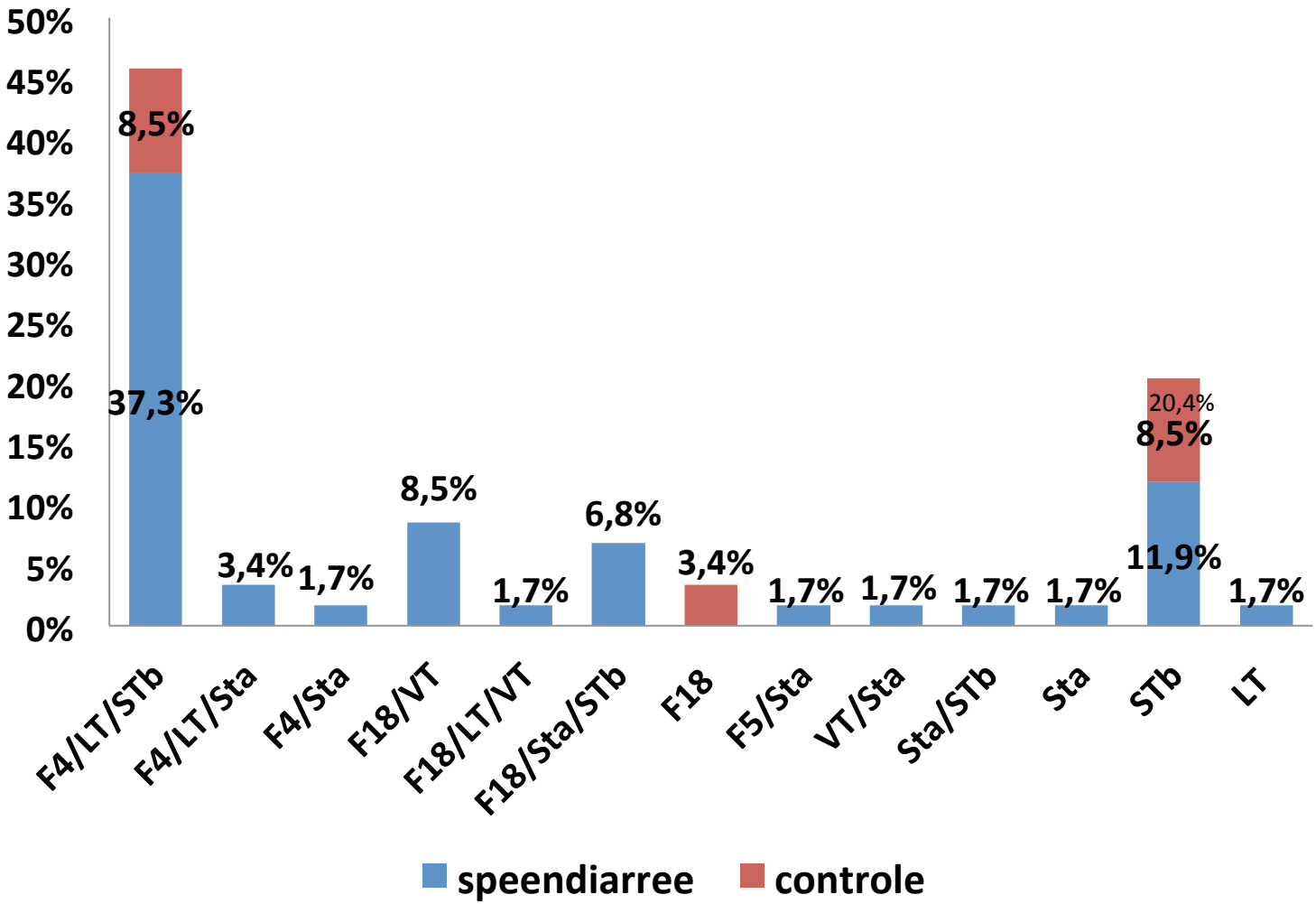
Enterotoxins (LT, STa, STb, EAST1)

Shiga-toxin (Stx2e)

Post-weaning diarrhoea

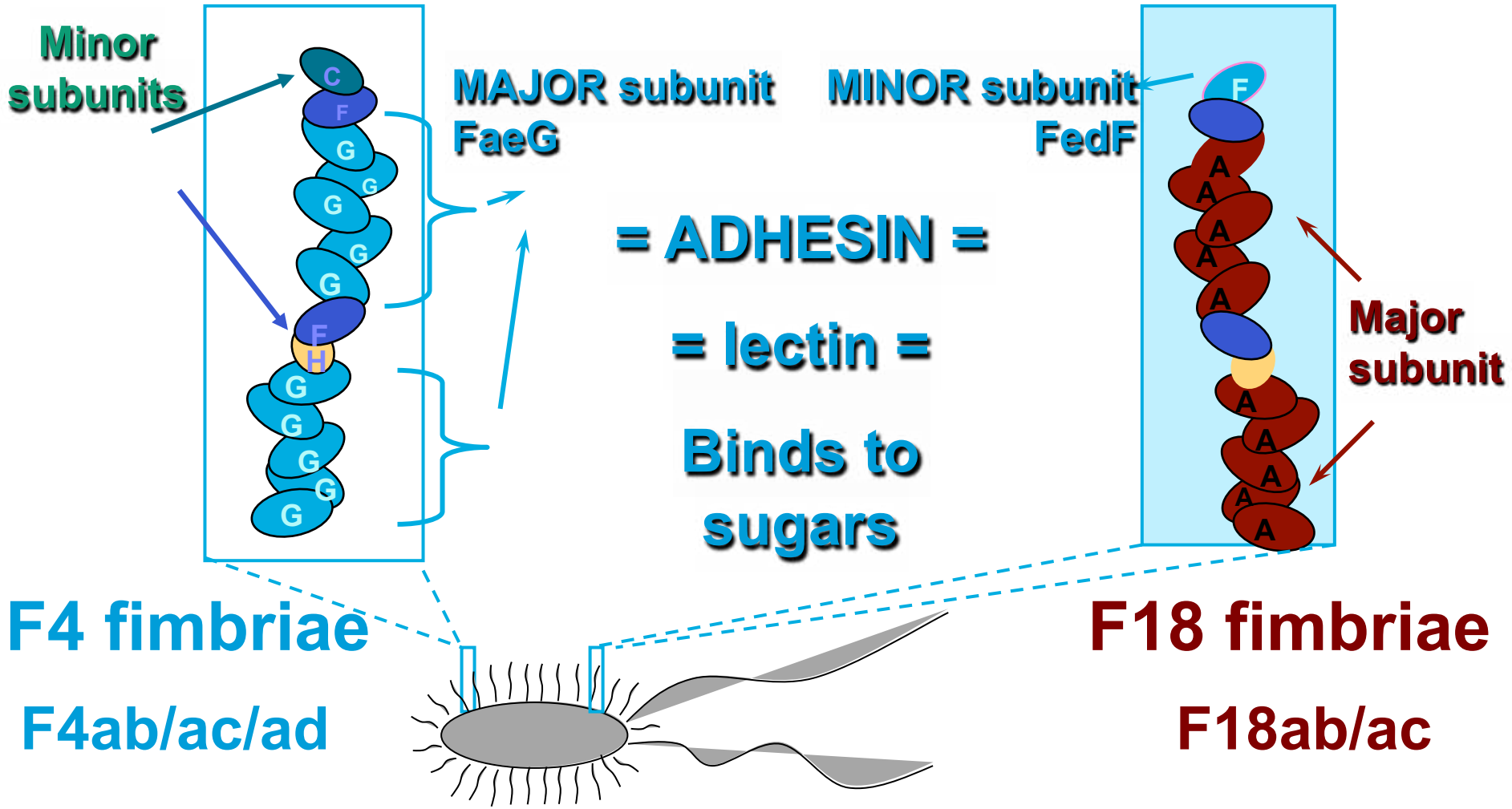
Oedema disease

E. coli virotypes post-weaning



Fimbriae
The colonisation factors

F4 and F18 differ in receptor-specificity



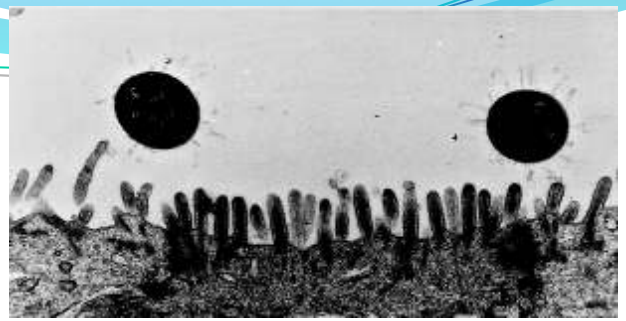
F4 receptor phenotype

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

Van den Broeck et al., Vet. Microbiol., 2000.

F4 binding is complex

Identified F4R



- Intestinal transferrin (GP74): F4ab

(Grange and Mouricout, 1996)

- Intestinal mucin-type sialoglycoprotein (IMTGP(s)): F4ab and F4ac, but not F4ad

(Francis et al., 1998)

- Intestinal glycosphingolipid (IGLad): F4ad

(Grange et al., 1999)

- Aminopeptidase N (ANPEP): F4ac

(Melkebeek et al., 2008)

- Galactosylceramide: F4ac

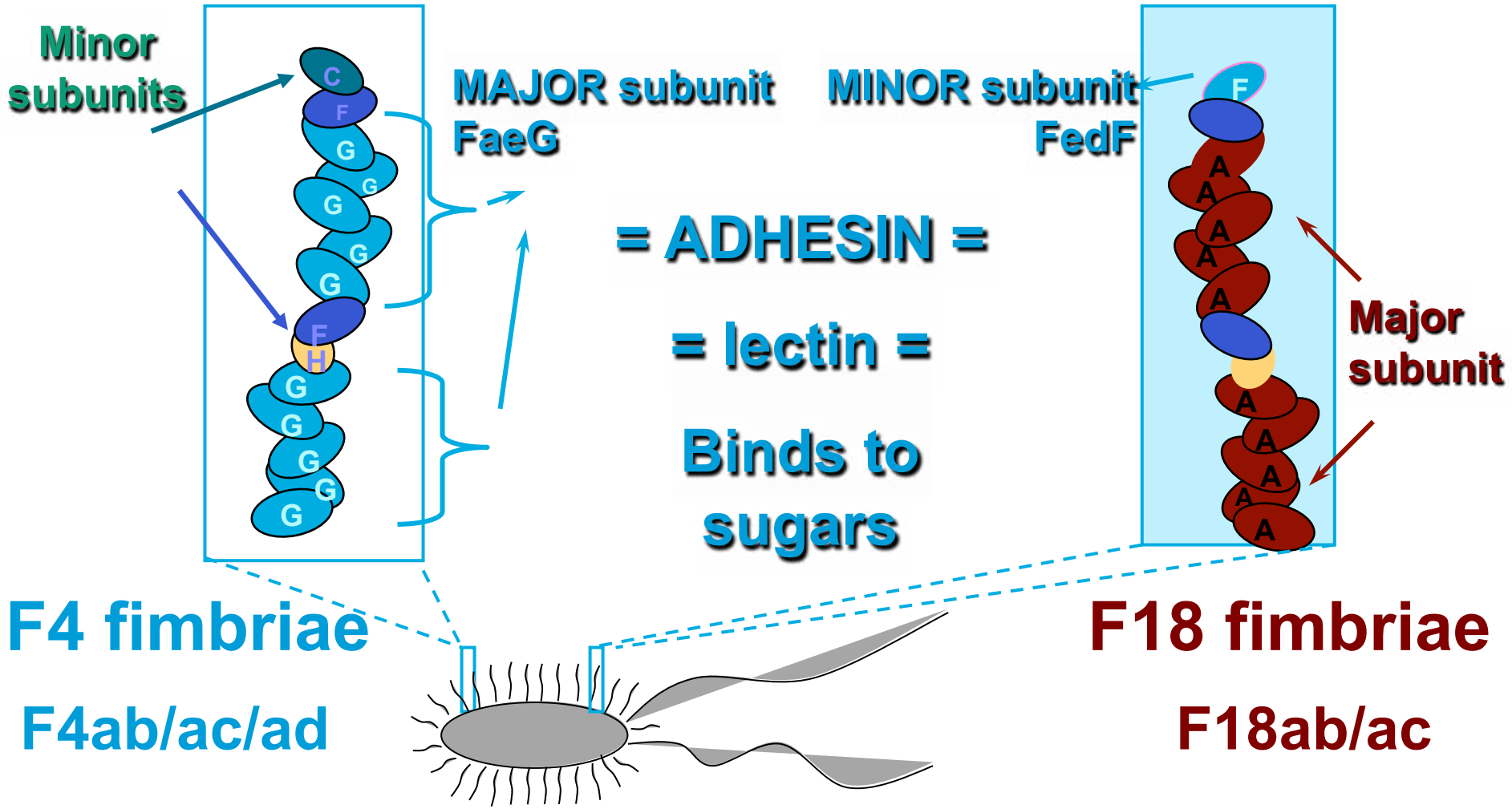
(Coddens et al., 2011)

- Sulfatide, sulf-lactosilceramide, Globotriaosylceramide: F4ab

(Coddens et al., 2011)

Sugars on these glycoproteins or glycolipids not identified

F4 and F18 differ in receptor-specificity



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

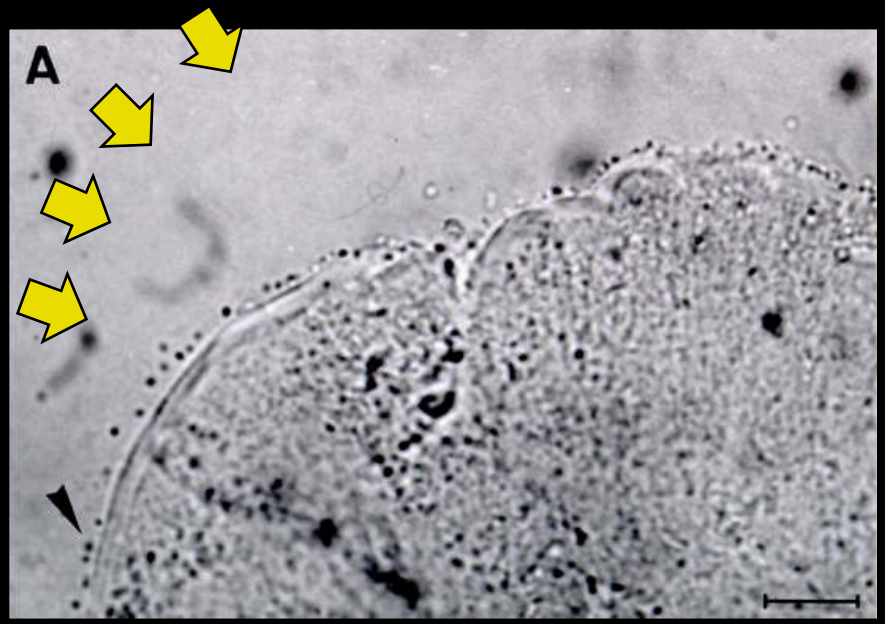
Tiels et al., Vet. Microbiol 2005

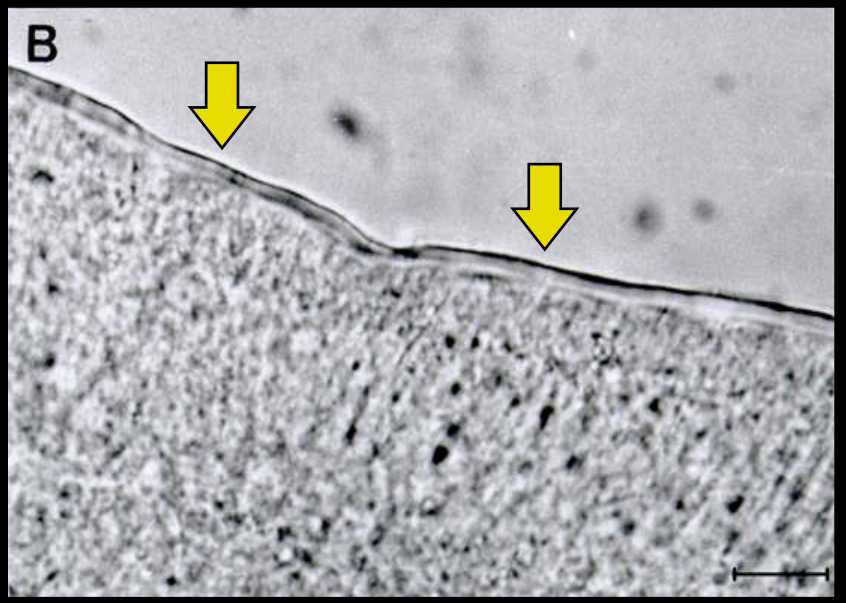
The expression of fimbrial receptors

Van Den Broeck et al., 1999.
Inf Imm

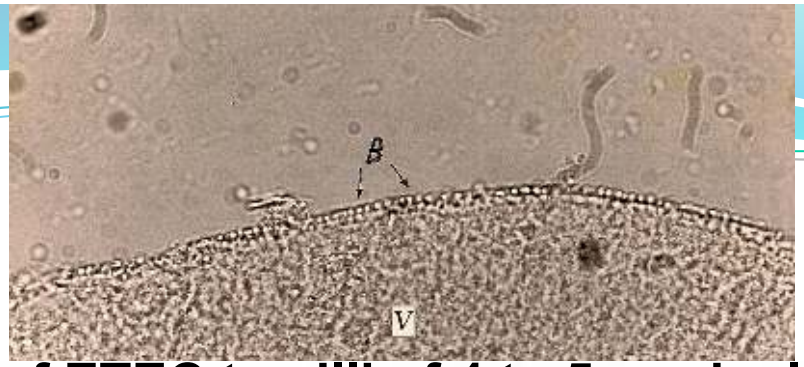
F4R +

F4R- (Natural knock-out)



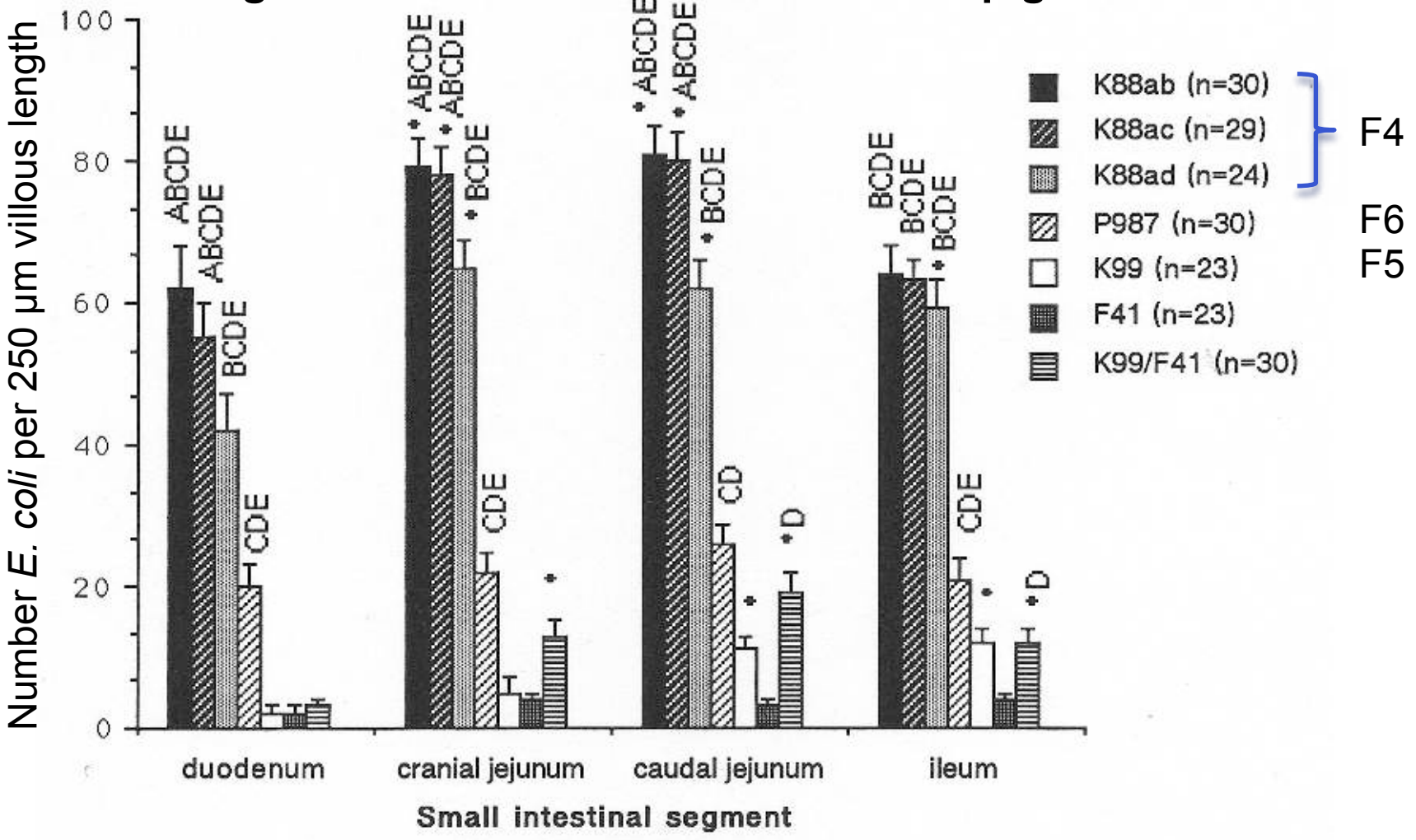


bar: 10 µm

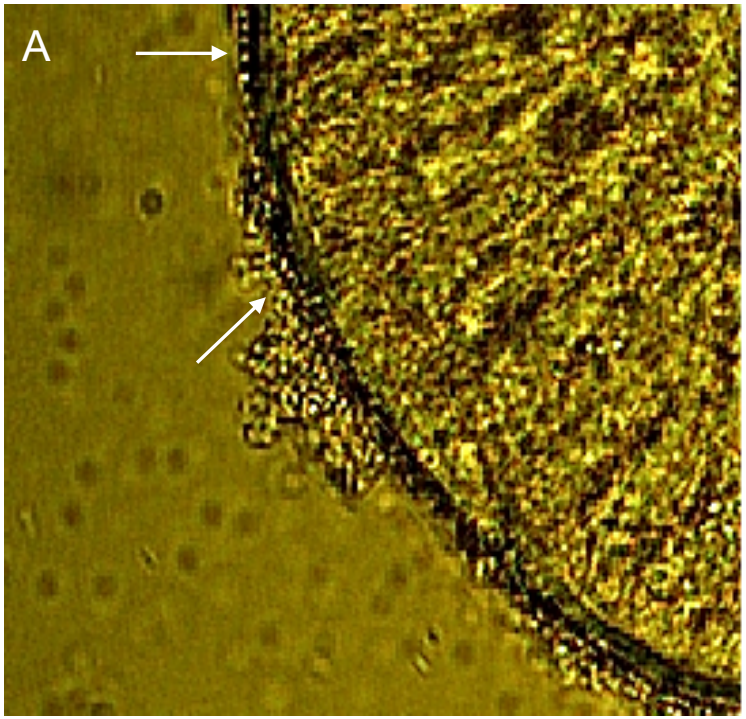


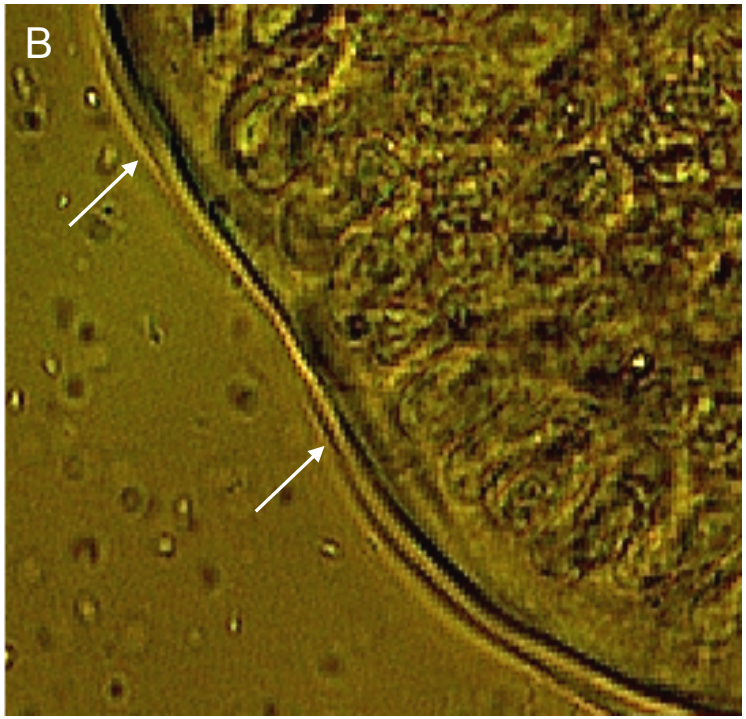
Cox et al., Vet. Microbiol., 1993

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



F18 receptors





In vitro villous adhesion assay

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

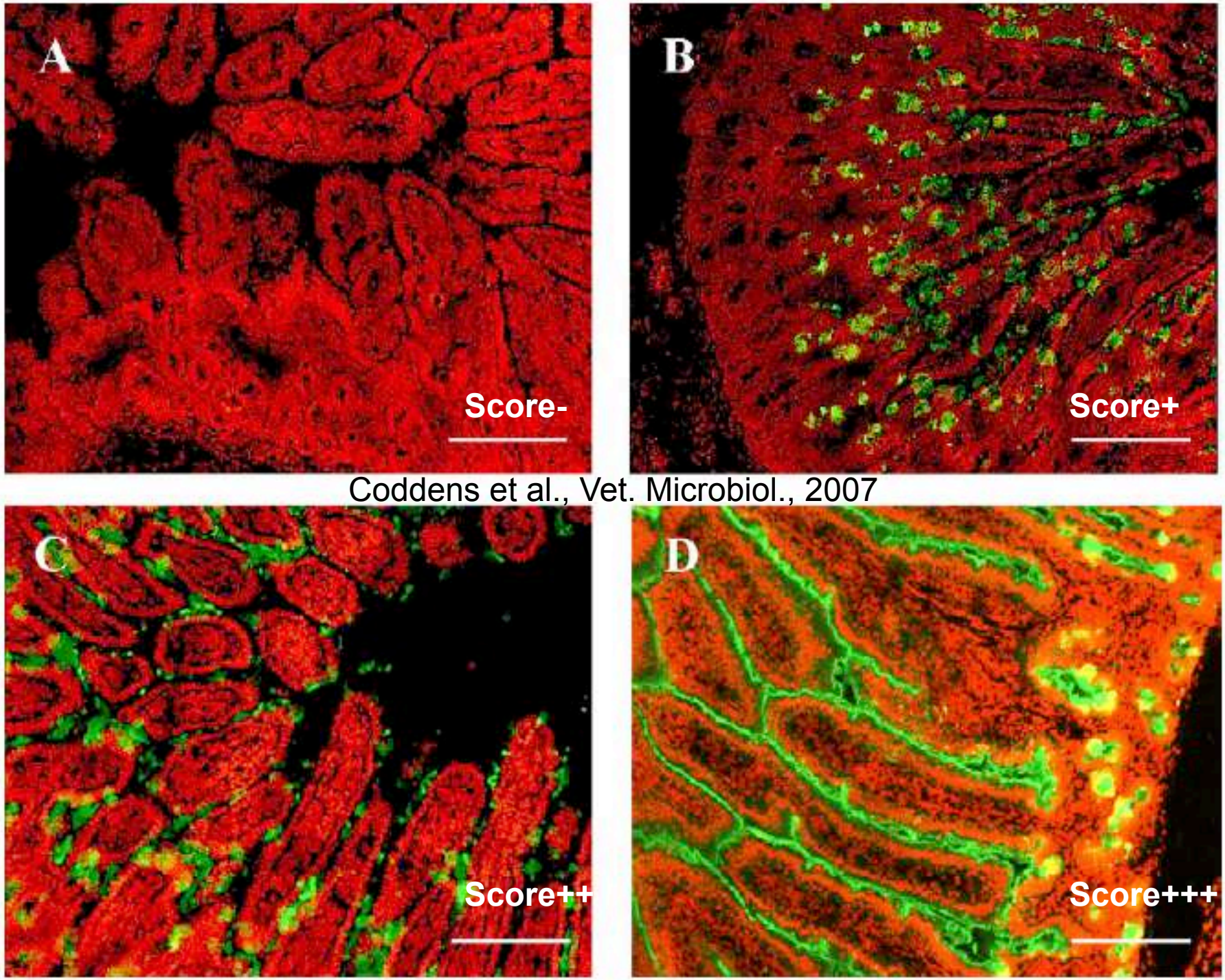
No adhesion ⇩ F18R negative piglets

Expression of F18R is function of age

Change in F18R expression with age was unkown: 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	G/A	A/A	<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 = resistant** Coddens et al., Vet. Microbiol., 2007



Coddens et al., Vet. Microbiol., 2007

Conclusion

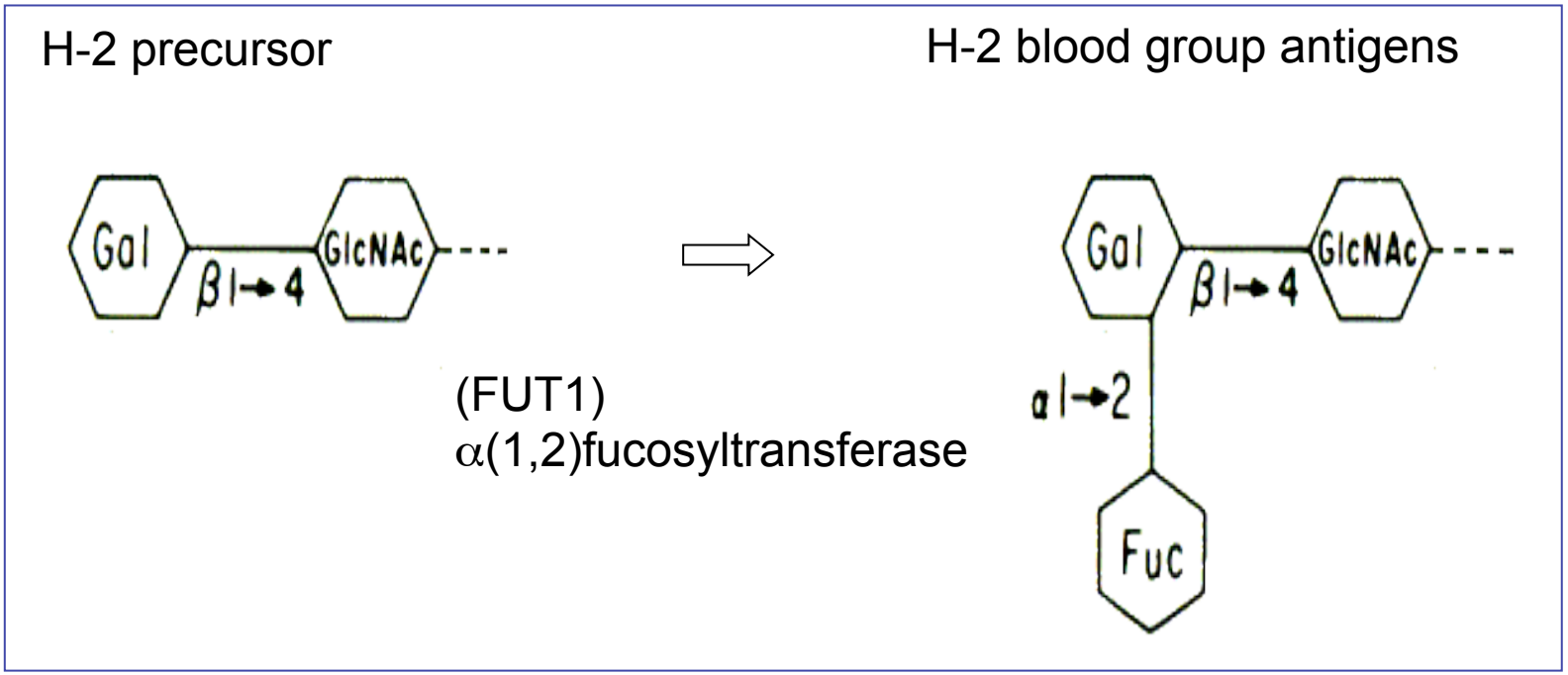
- Expression of the F4R is irrespective of age
- Expression of the F18R is age dependent
- There is sufficient F18R expression from 3 weeks of age

The genetic test for the F18 receptor

The genetic test for the F18R phenotype

***FUT1* gene** is associated with expression of the F18R

Codes for α(1,2)fucosyltransferase → **Blood group AO antigens**



Meijerink et al., 1997, 2000

The genetic test for the F18R phenotype

- Sequencing of ***FUT1*** of F18R- en F18R+

Located in the halothane linkage group on pig chromosome 6

➡ Polymorphism at base pair 307 of *FUT1 gene*:
Transition of Guanine->Adenine

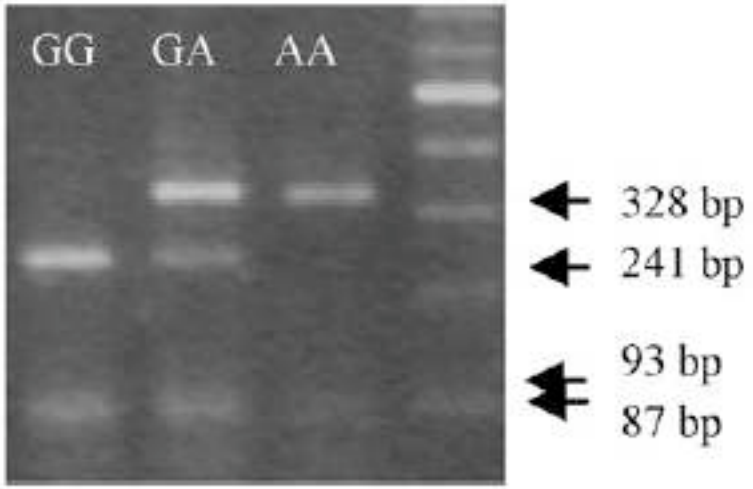
➡ GG, GA => F18R+
AA => F18R -

- **Blood group antigens are**
  - Expressed on erythrocytes
  - Expressed on epithelial cells (intestine, urinary tract)
  - Secreted (saliva, milk)

Meijerink et al., 1997, 2000

F18R

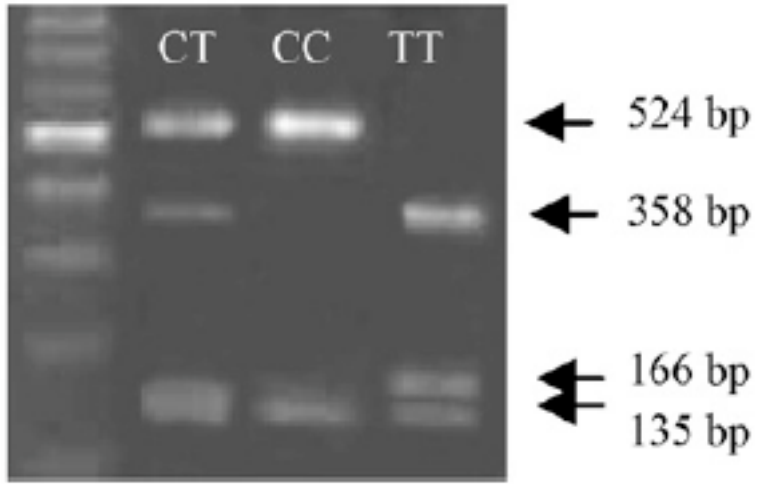
F18R+ F18R+ F18R-



Digestion of an amplified FUT1 fragment with the restriction enzyme CfoI

Stress (halothane test)

\- - +



Digestion of an amplified RYR1 fragment with the restriction enzyme Alw21I

Correlation between F18R expression and stress susceptibility

	FUT1	RYR1		
		CC ($n = 88$)	CT ($n = 40$)	TT ($n = 3$)
F18R+	GG ($n = 82$)	58	23	1
F18R+	GA ($n = 43$)	26	15	2
F18R-	AA ($n = 6$)	4	2	0
		-	-	+

Stress

Coddens et al., Vet. Microbiol., 2008

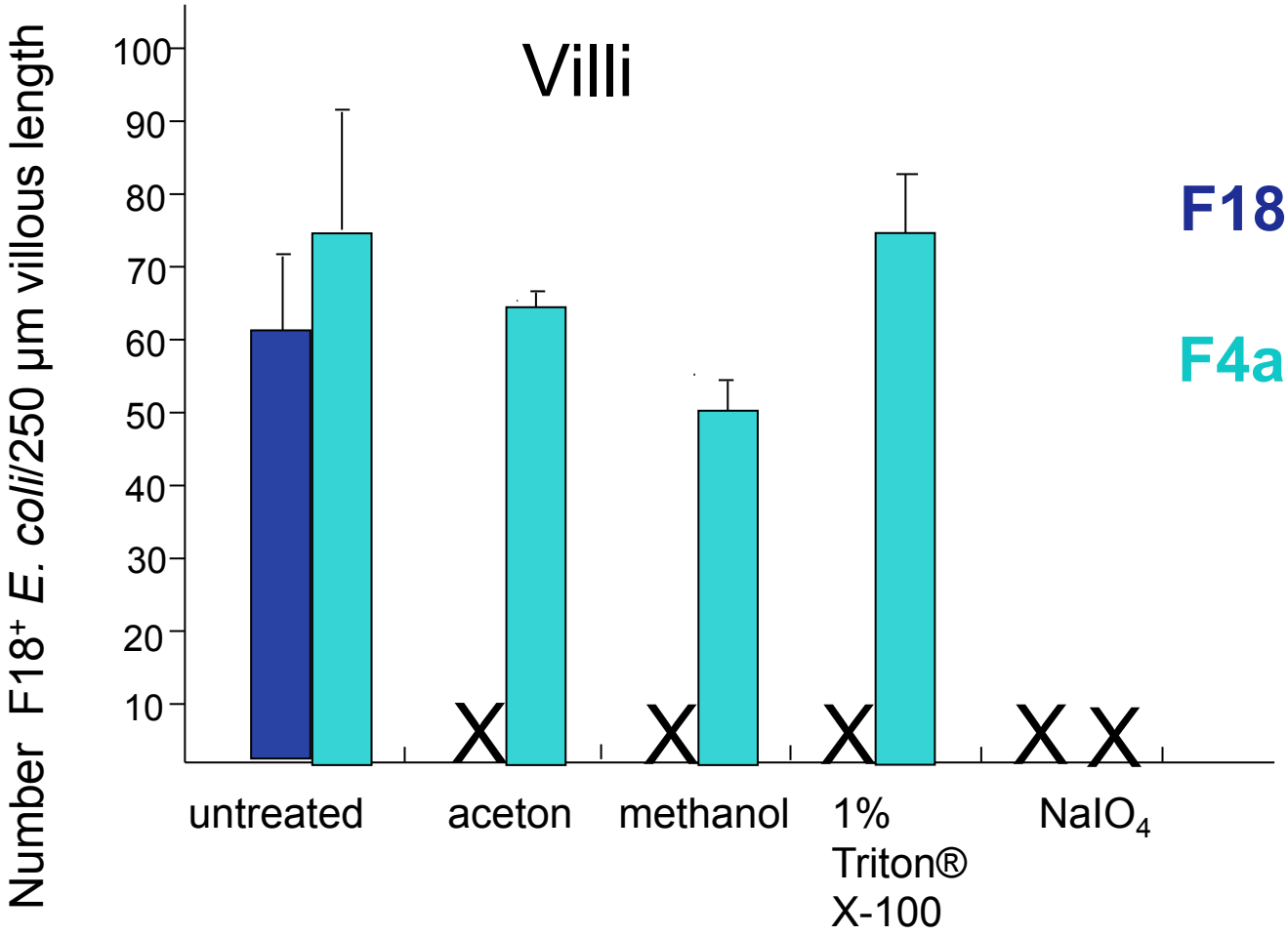
Conclusion

It is possible to select pigs for absence of the F18R and stress resistance

A test for F4ab/acR negative piglets is nearby

Nature of the F18 receptor

Determining the nature of the F18R



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

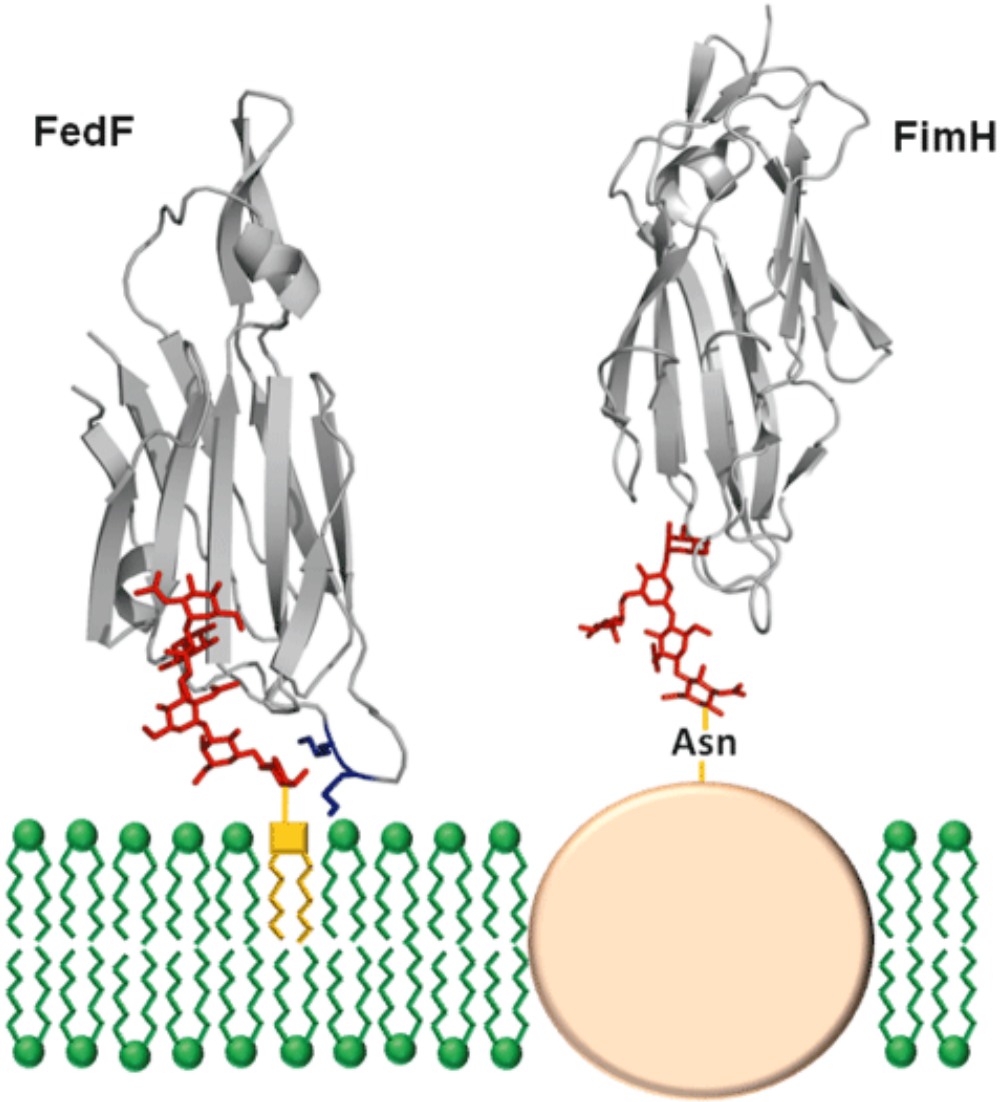
F4ac+ *E. coli* (n = 2)

⇒The F18R is a glycolipid

⇒The F4R is a glycoprotein

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

Structural insight in binding of the F18 fimbrial adhesin FedF to blood group A6 type1



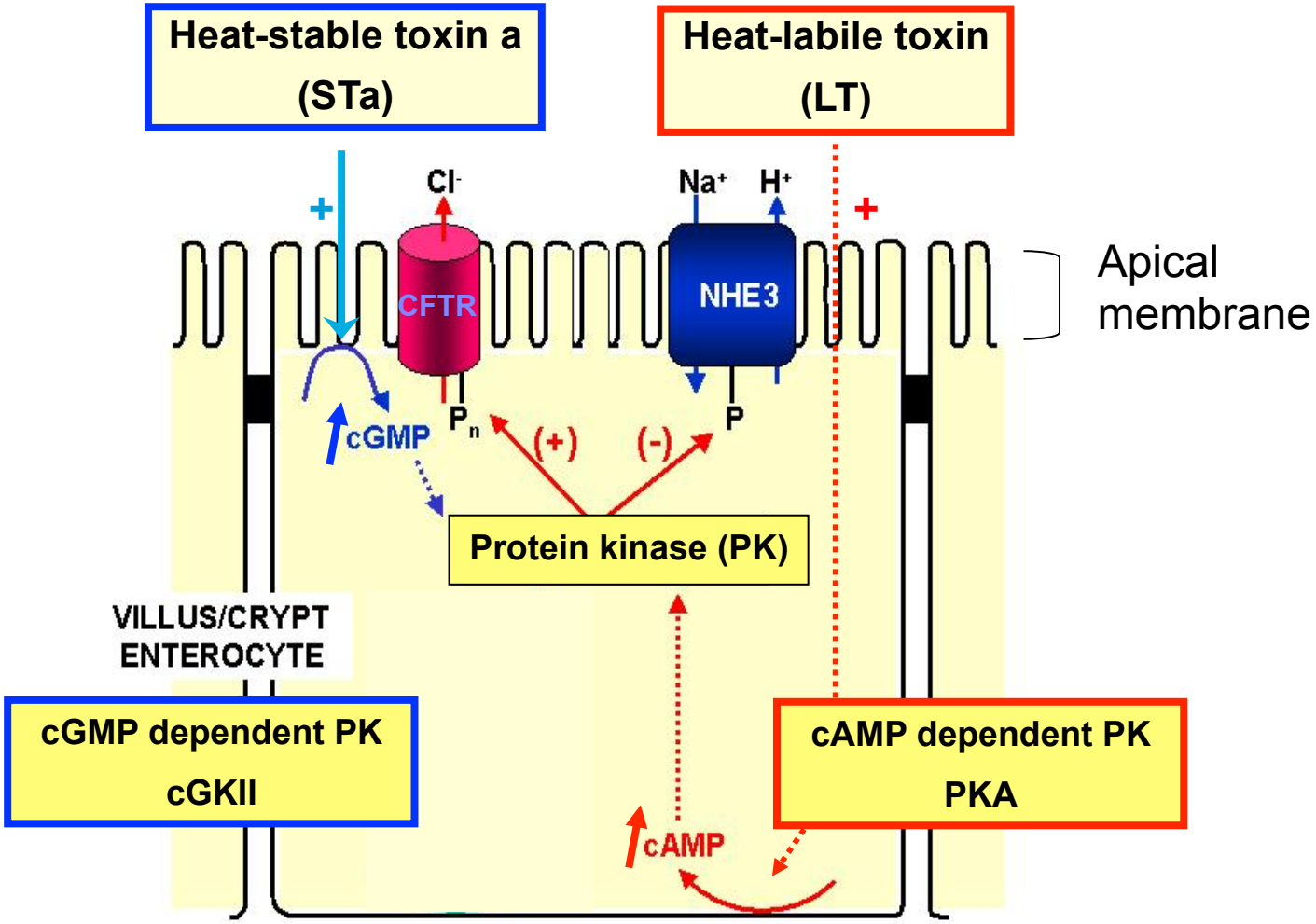
Moonens et al., 2012. Molecular Microbiology, 86, 82-95.

Conclusion

F18 adheres to blood group A and O sugars

Enterotoxins induce diarrhoea

PATHOGENESIS: ENTEROTOXINS

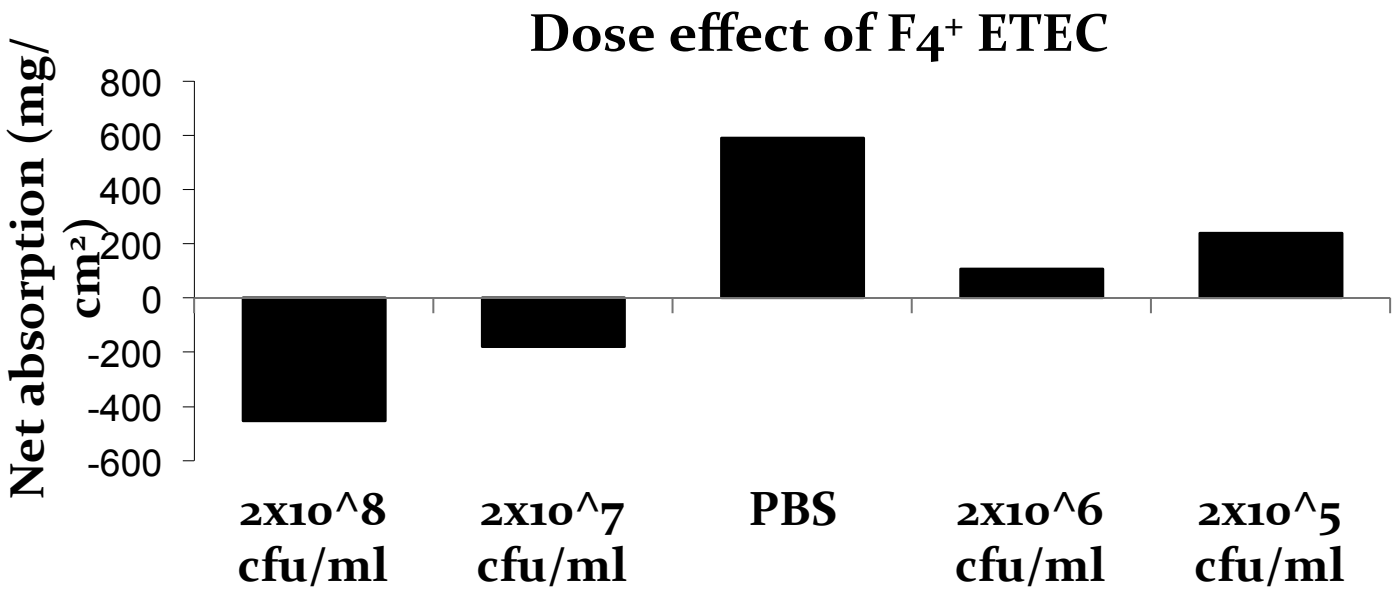


Heat-stable STb → sulfatide → Ca^{2+} → prostaglandin E_2, serotonin

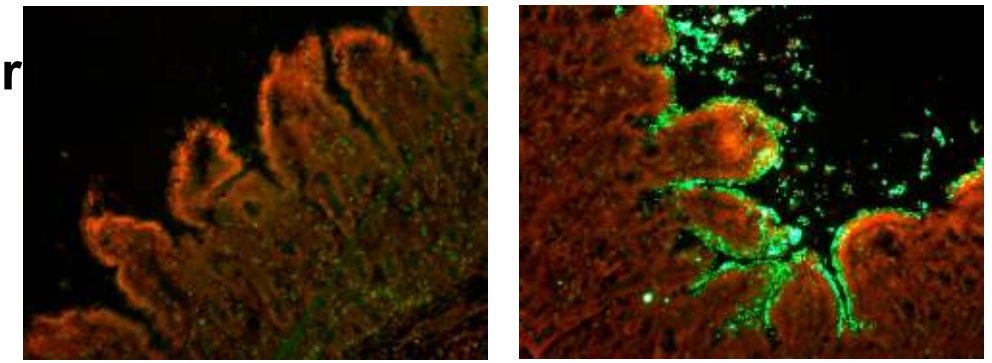
luminal electrolytes ↑→ water excretion → diarrhea?

GIS26= F4+ ETEC LT+STA+STB+

F4R+ pig 8h perfusion of small intestinal segment



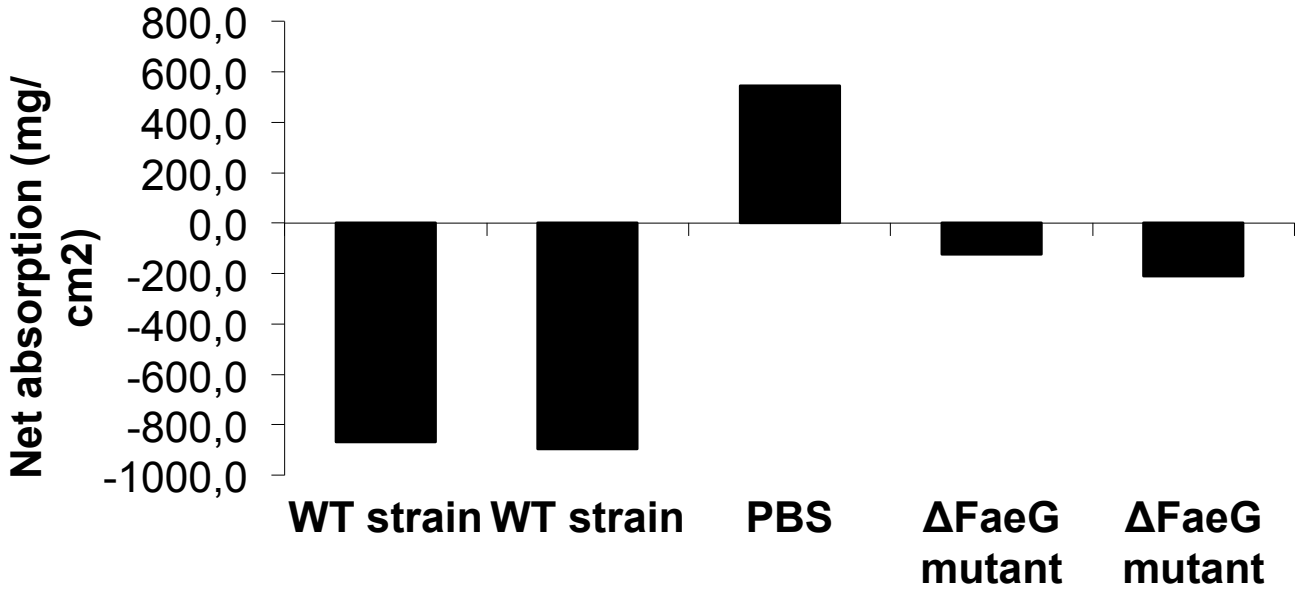
Adhesion of the F4+bacter



PBS ETEC GIS26

IMPORTANCE OF F4 MEDIATED ADHESION

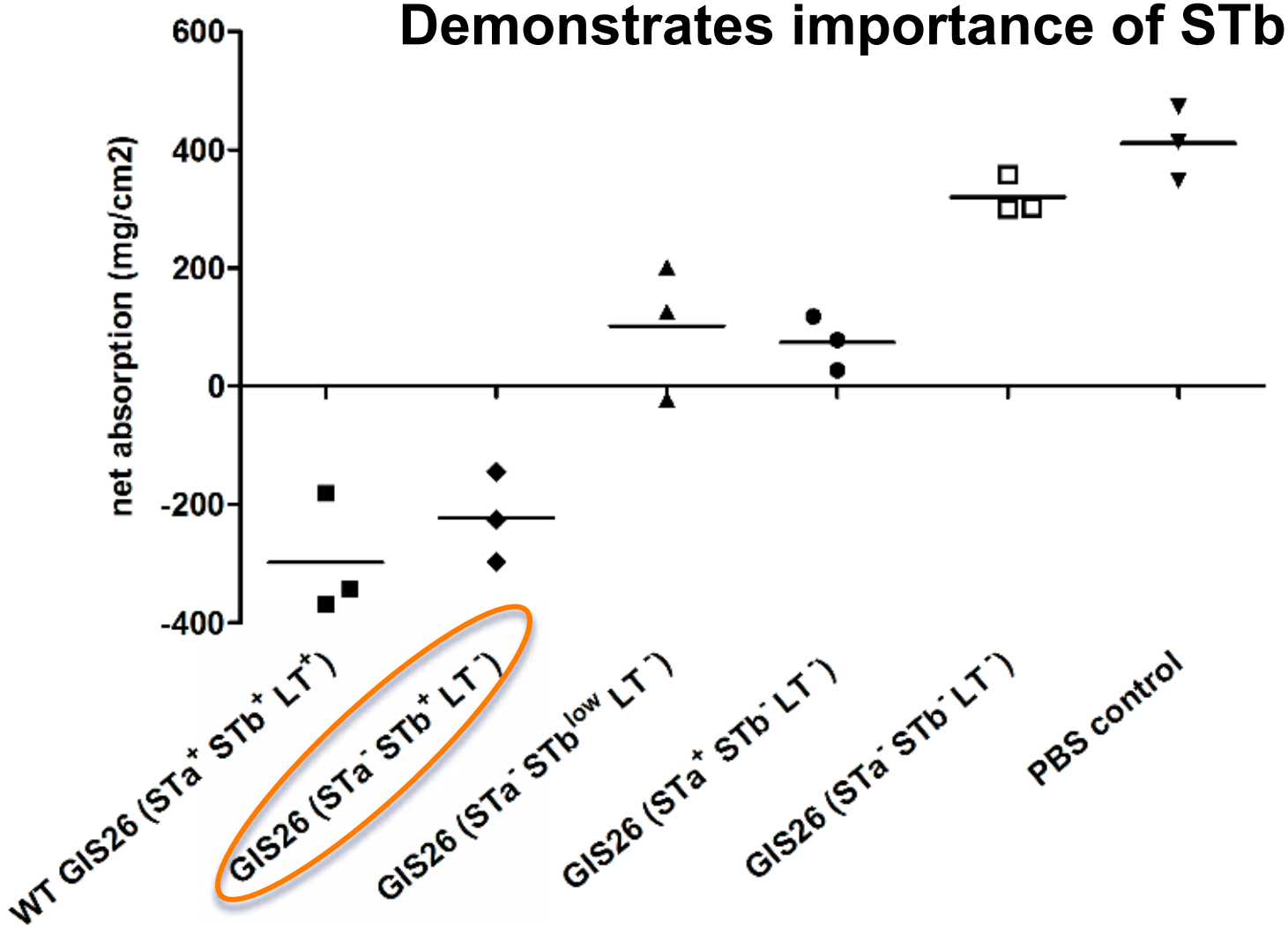
- F4R$^+$ pig, **8h** perfusion
- Compare wild type with mutant strain lacking F4 (deletion of FaeG subunit)



F4 mediated adhesion not necessary but stronger effect

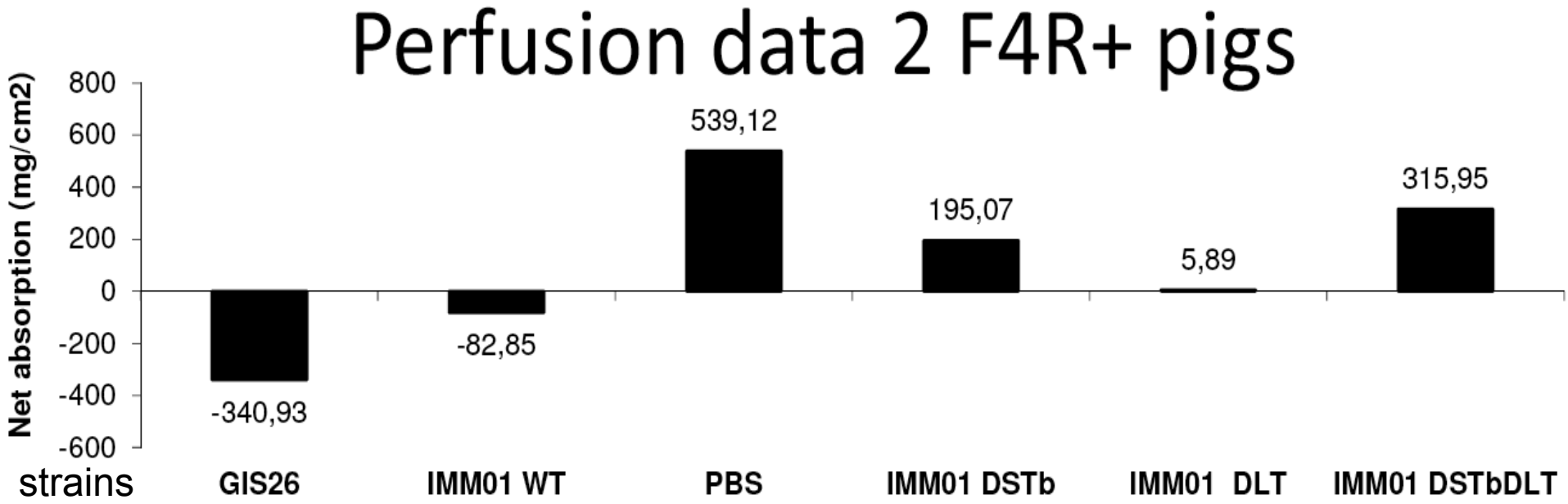
Small intestinal segments were infected and subsequently perfused during 4 hours Demonstrates importance of STb

N = 3 F4R+



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

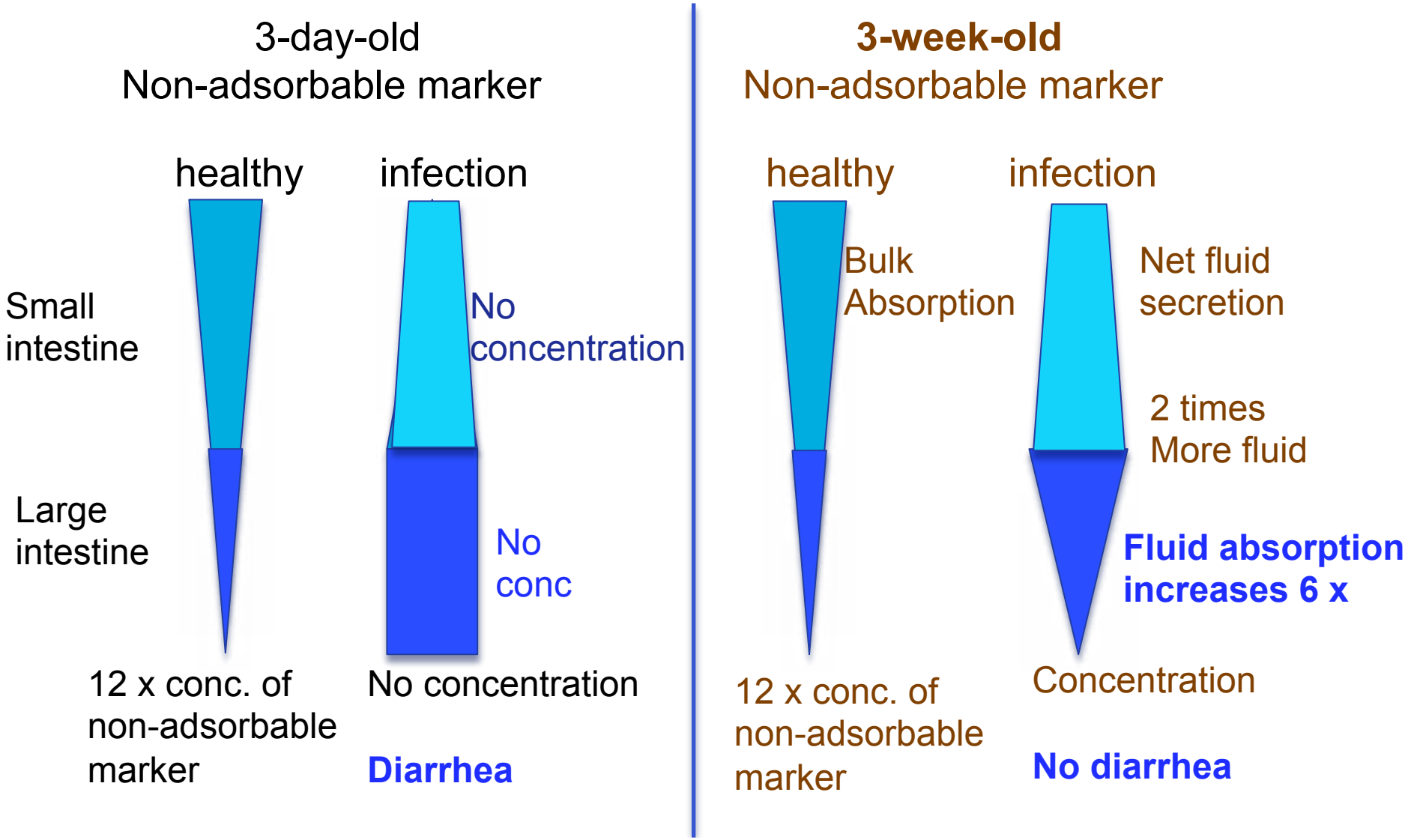
Effect of wild type and mutant IMM01 ETEC strains on net fluid absorption (mg/cm^2) in 7h-infected jejunal segments of F4R positive pigs



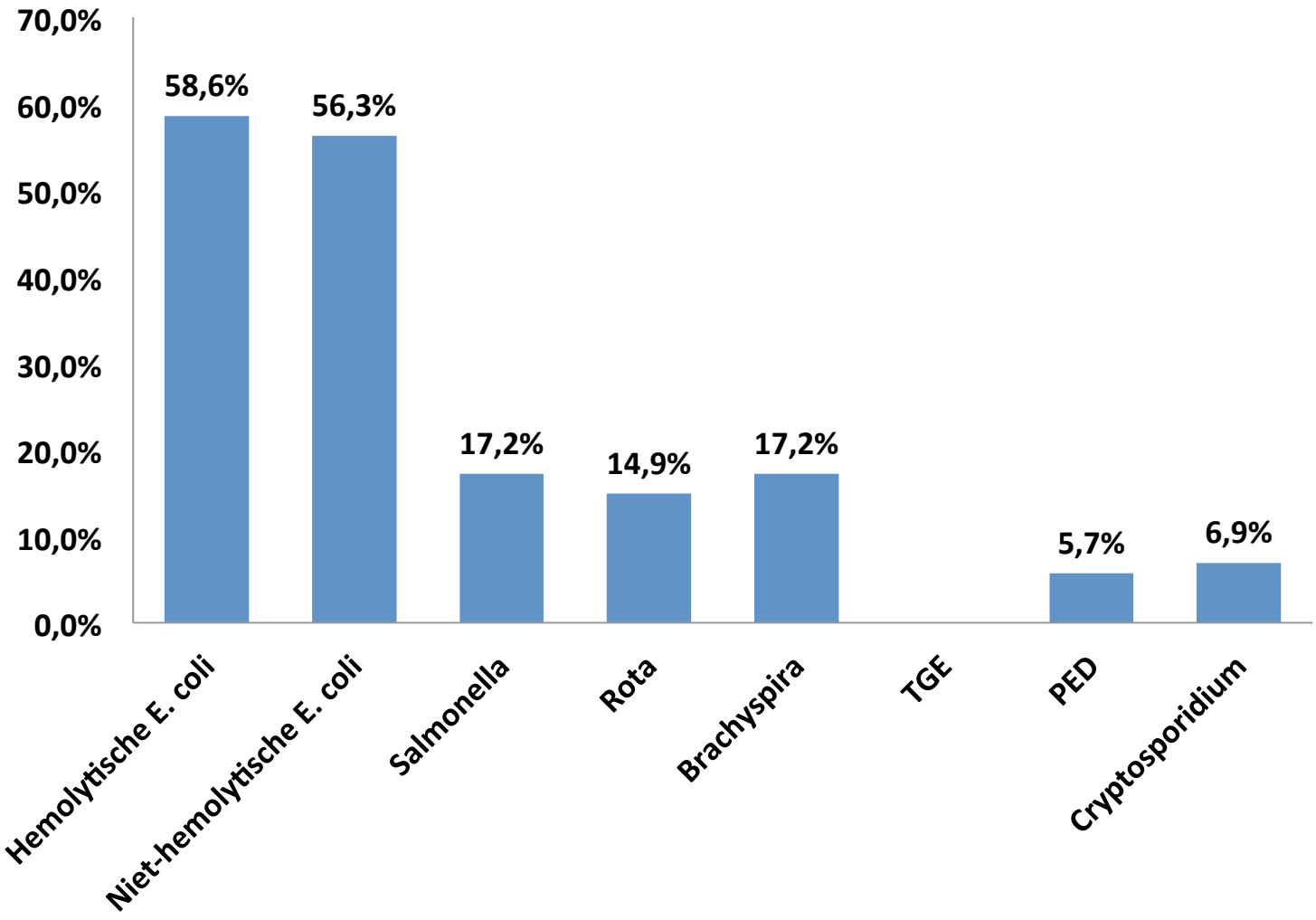
toxins	LTSTaSTb	LTSTb		LT	STb	no

Argenzio et al, 1984

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



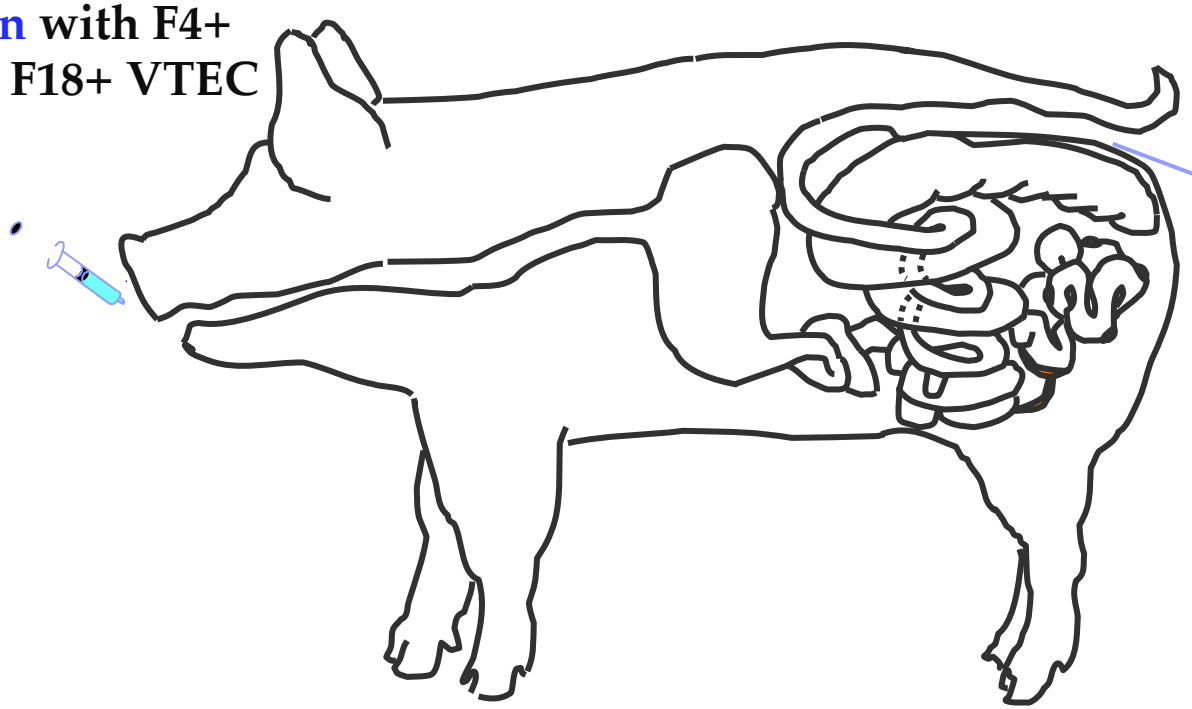
Bacteria and viruses identified in faeces of pigs postweaning on Belgian farms



Infection and antibody response

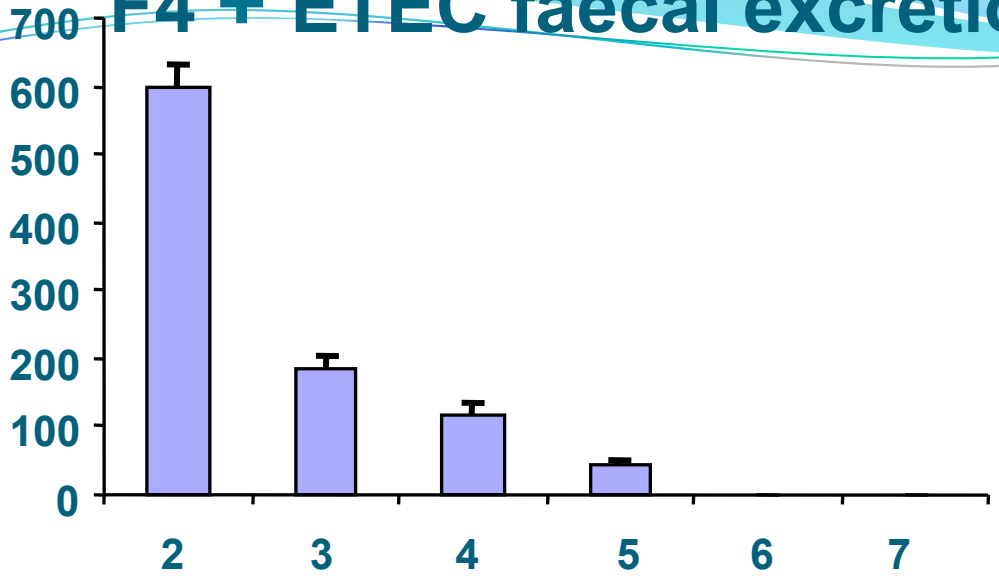
Infection of weaned pigs with ETEC or VTEC

Oral infection with F4+ ETEC or F18+ VTEC



Faecal excretion

F4 + ETEC faecal excretion

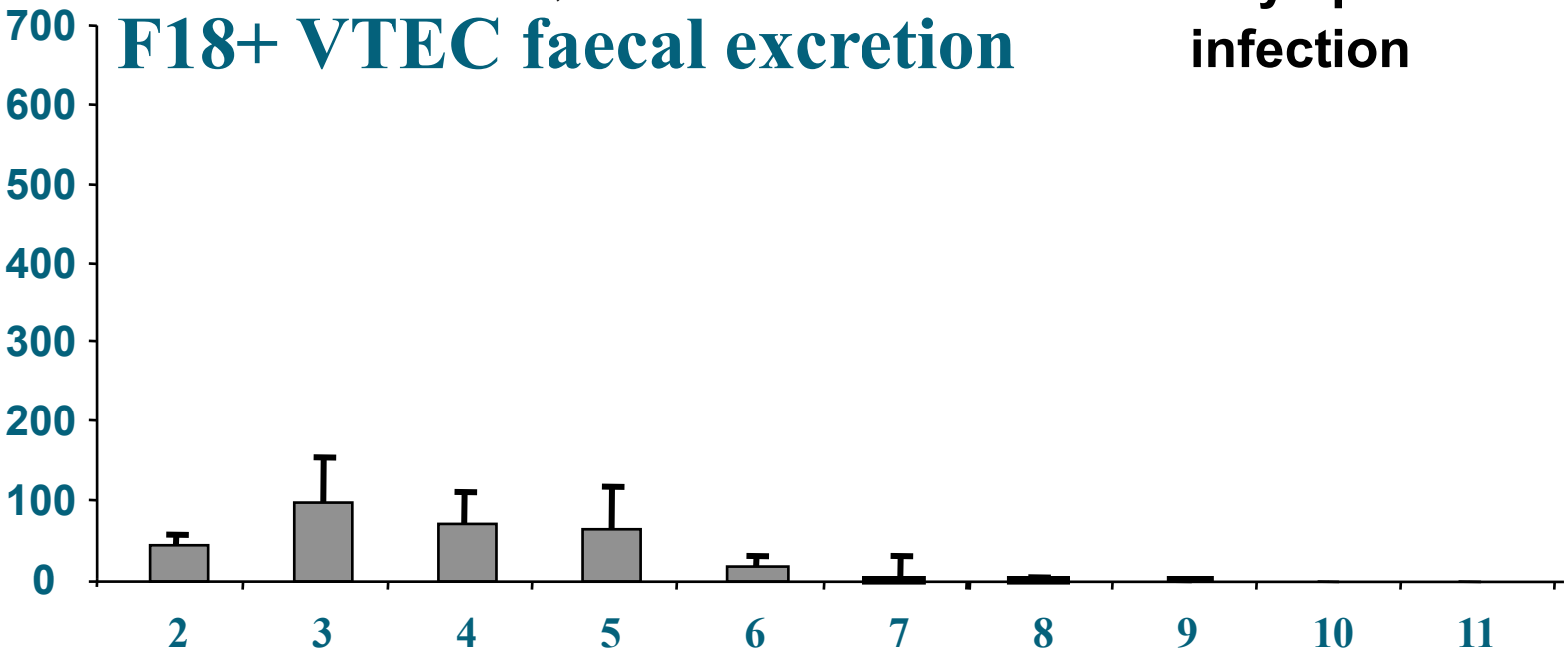


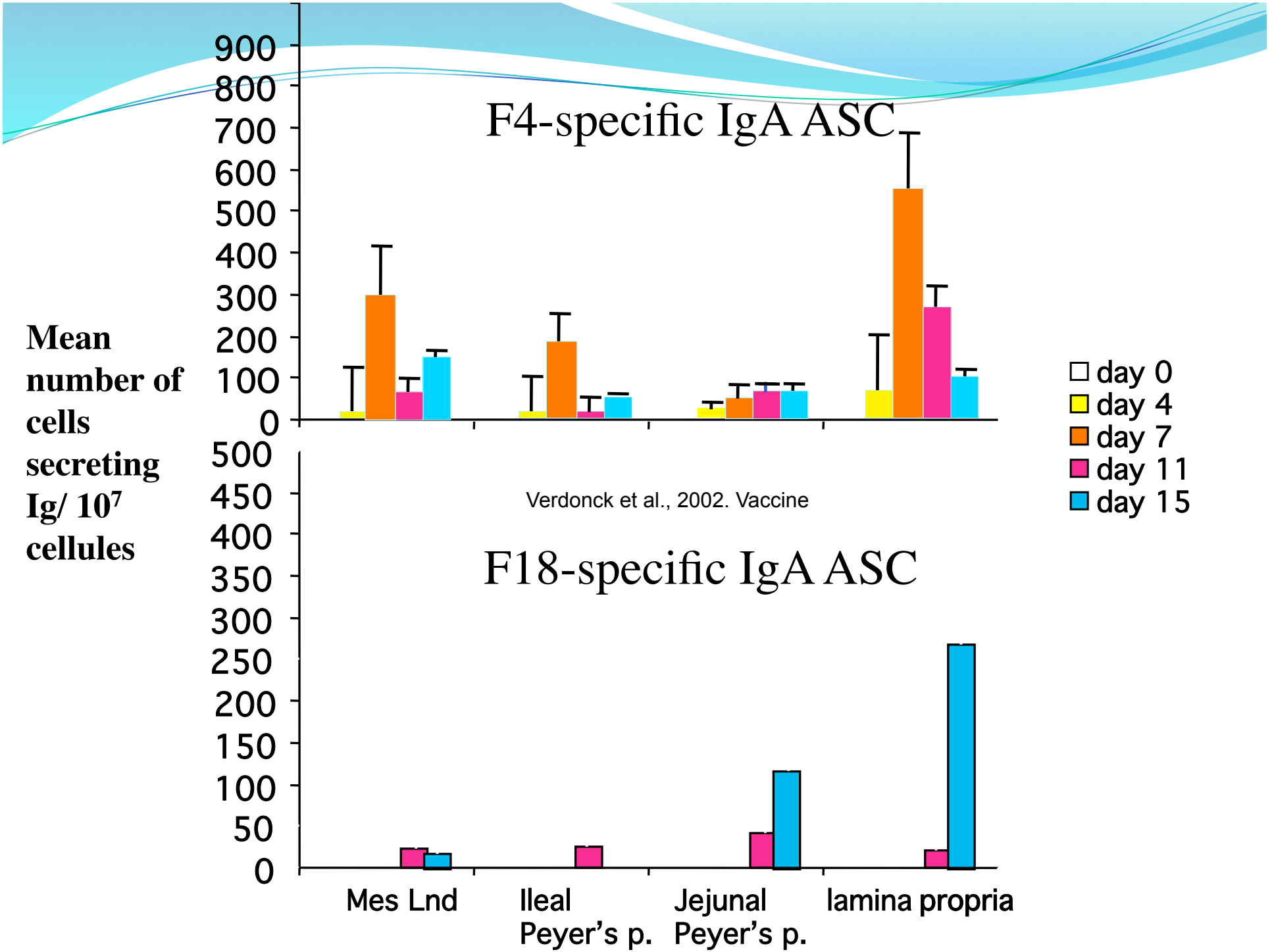
10^6 *E. coli* per g faeces

Verdonck et al., 2002. Vaccine

Days post infection

F18+ VTEC faecal excretion




F4-specific IgA ASC
Mean number of cells secreting Ig/ 10⁷ cellules
900
800
700
600
500
400
300
200
100
0
day 0
day 4
day 7
day 11
day 15
Verdonck et al., 2002. Vaccine
F18-specific IgA ASC
500
450
400
350
300
250
200
150
100
50
0
Mes Lnd
Ileal Peyer's p.
Jejunal Peyer's p.
lamina propria

Conclusion

Infection with F4+ ⟺ F18+ *E. coli*

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

How can we tackle these infections?

- Vaccination?
  - Oral against the fimbriae
    - dead
    - live
  - Systemic against the toxin
- Genetic selection?

Protection small intestine

Virulence

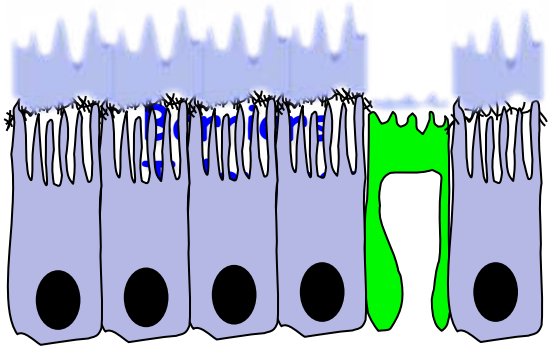
VACCINE

alive ⟷ dead

Too attenuated => no danger

Too virulent => disease

Reversal of virulence by plasmids



Intestinal Mucosa

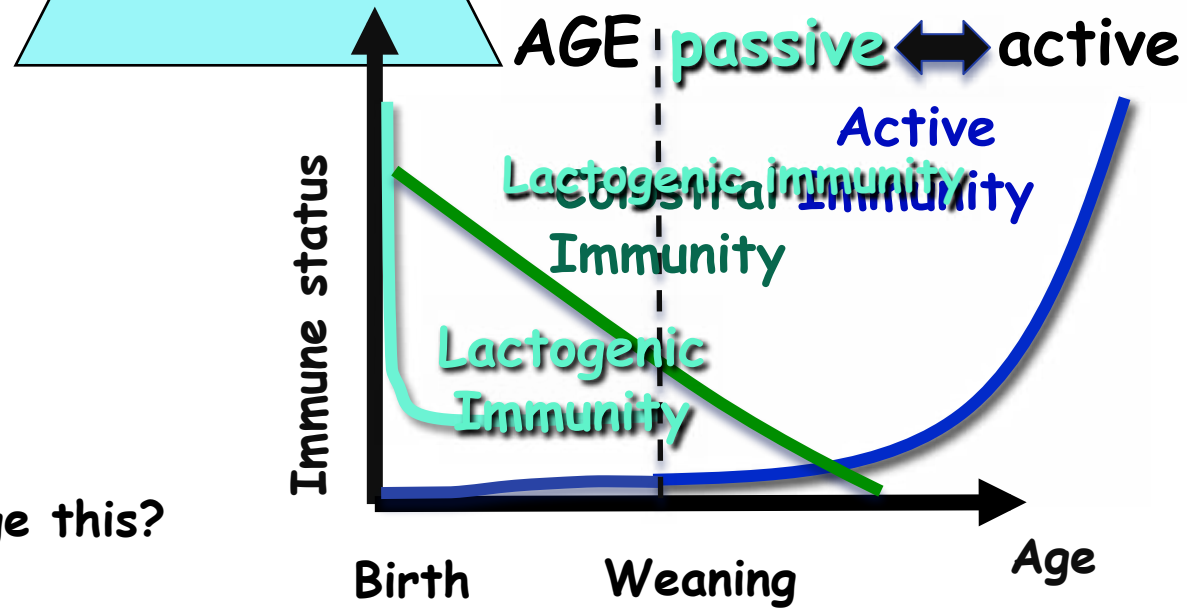
ROUTE

parenteral ⟷ mucosal

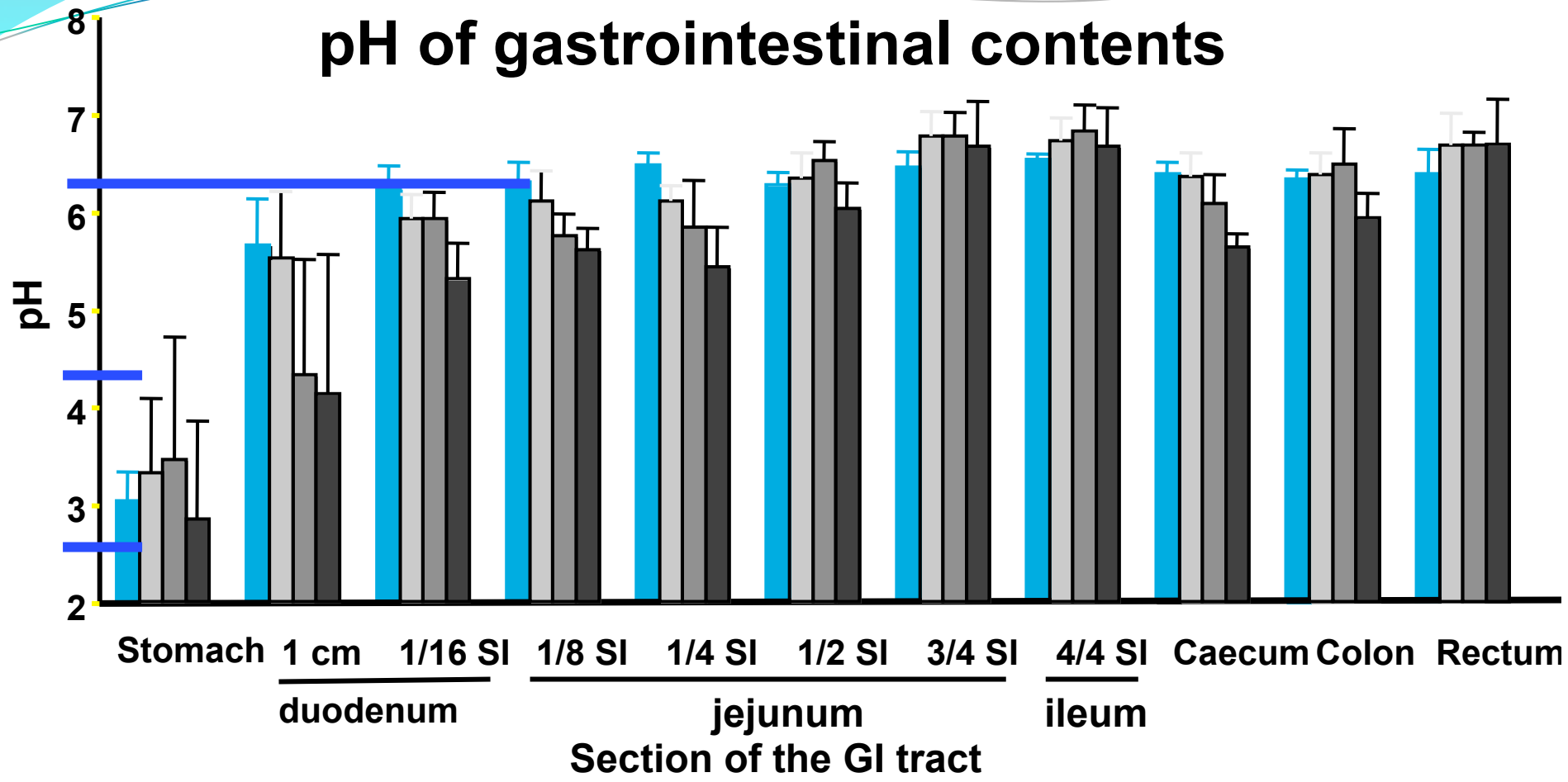
Oral preferred route

Not mucosa

Can immunomodulation change this?



pH of gastrointestinal contents



Suckling piglets n=6

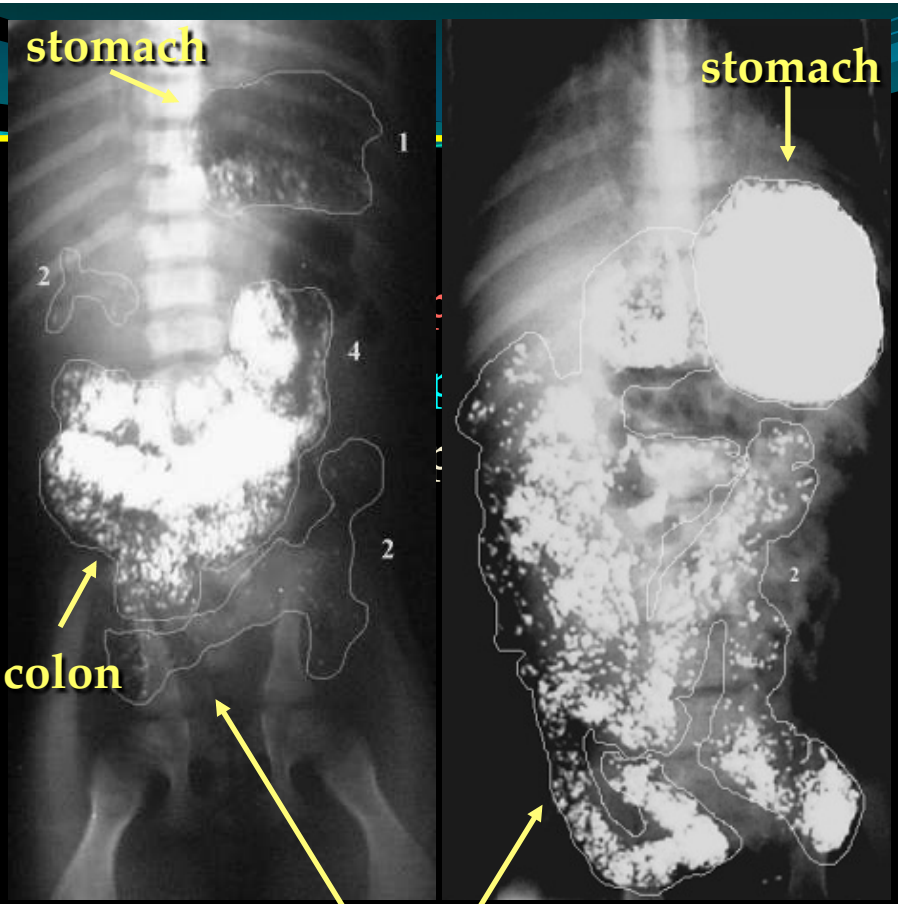
3 days postweaning n=6

1 week postweaning n=6

2 weeks postweaning n=6

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

Gastric emptying



Suckling
3 weeks PW
2 weeks PW
3 days postweaning

% pellets in the stomach

100
80
60
40
20
0

75% emptied

0 10 20 30 40 50 60 70 80

< 3.5 h
7 h
± 15 h
± 30 h

Time post administration (h)

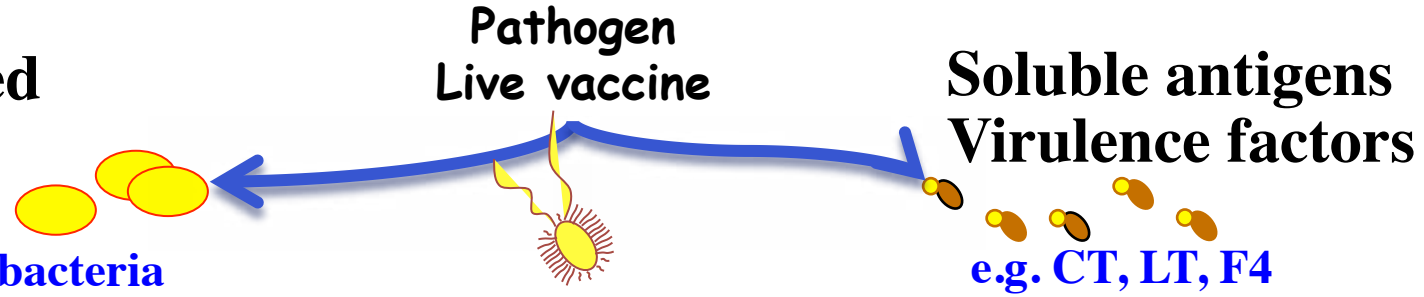
Snoeck et al., 2004. Journal of Controlled Release 94, 143– 153

Oral vaccination remains difficult

Follicle-associated epithelium

M-cells

Enterocytes



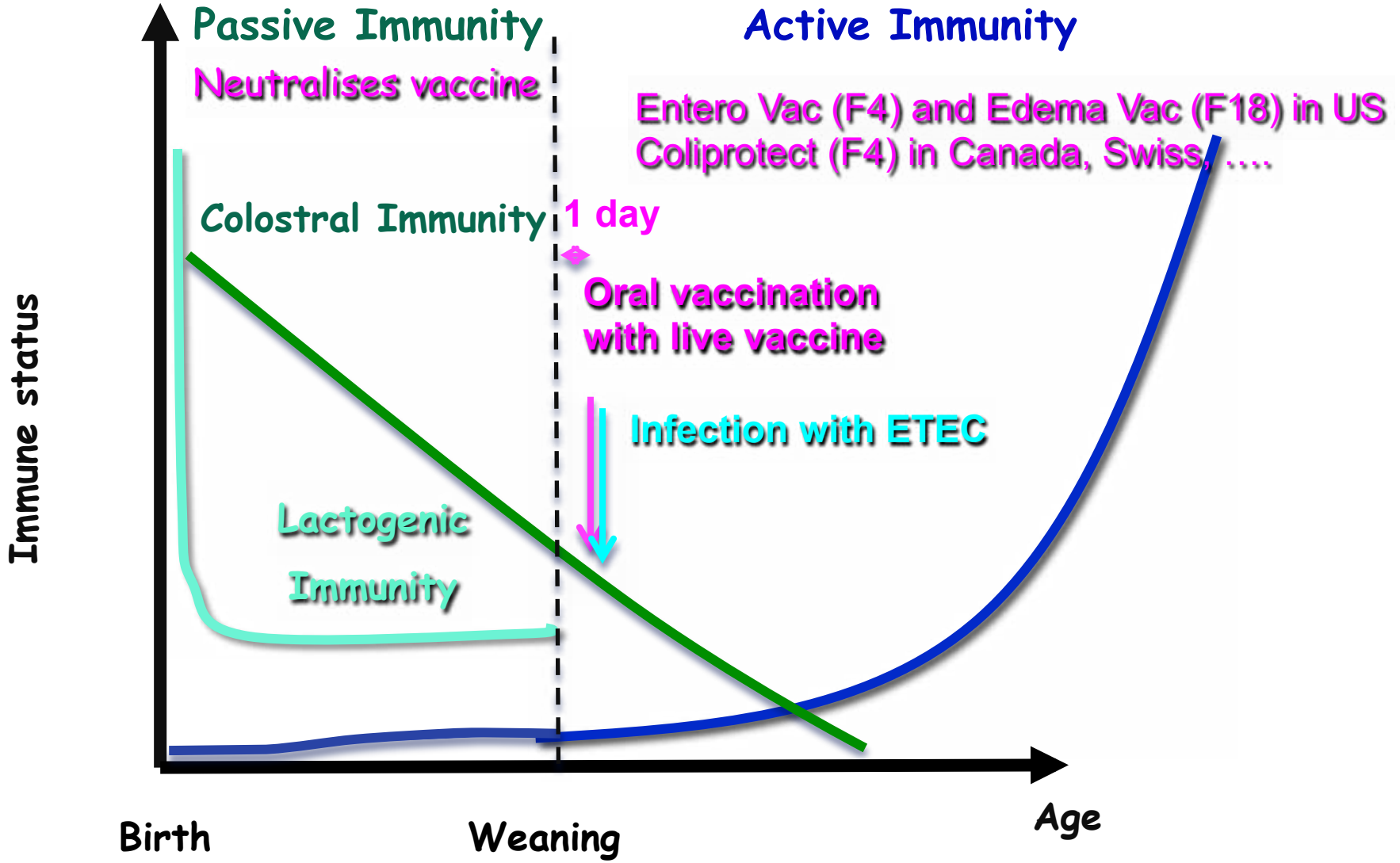
Selection of antigens

- Antigens remain immunogenic (production process)
- Reach FAE (target)
- Transcytose by M cells (target?)
- Danger signals (adjuvant?)
- Uptake by DCs
- Maturation of DCs

- Antigens retain immunogenic (protection)
- Reach enterocytes (target?)
- Transcytose by enterocytes (target?)
- Danger signals (adjuvant?)
- Uptake by DCs
- Maturation of DCs

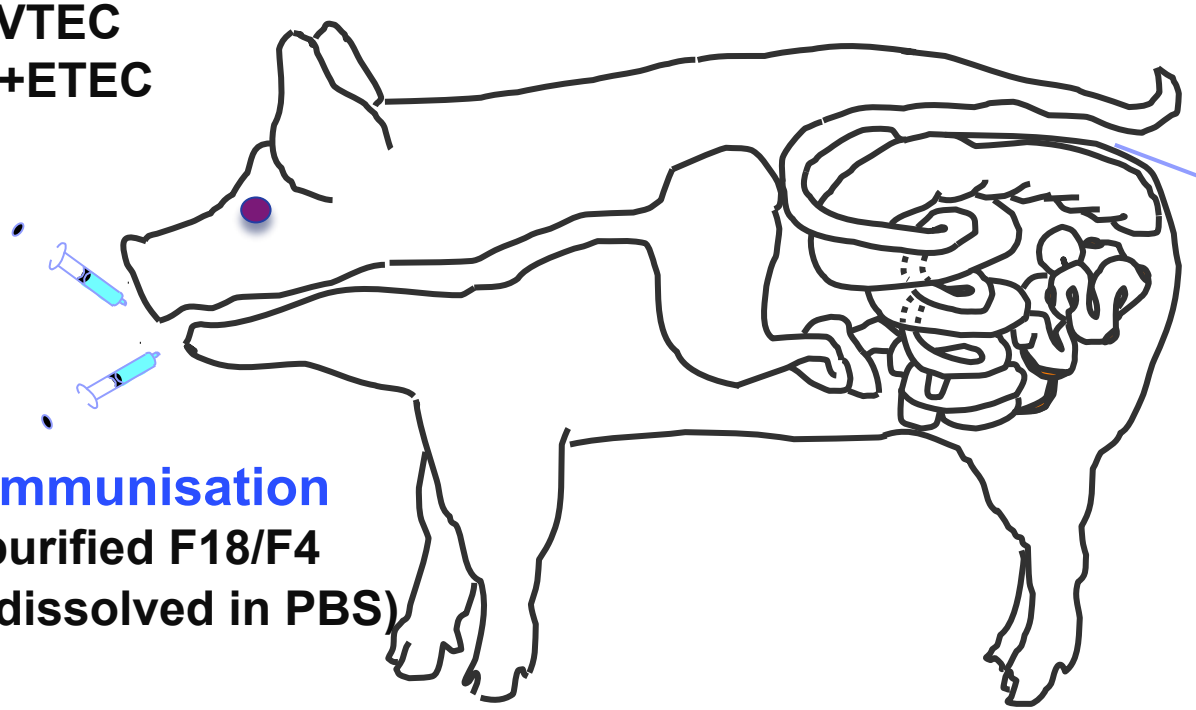
Immunity

Challenge in vaccination against post-weaning diarrhoea is the passive immunity



Immunization with F4 or F18 fimbriae via the oral route

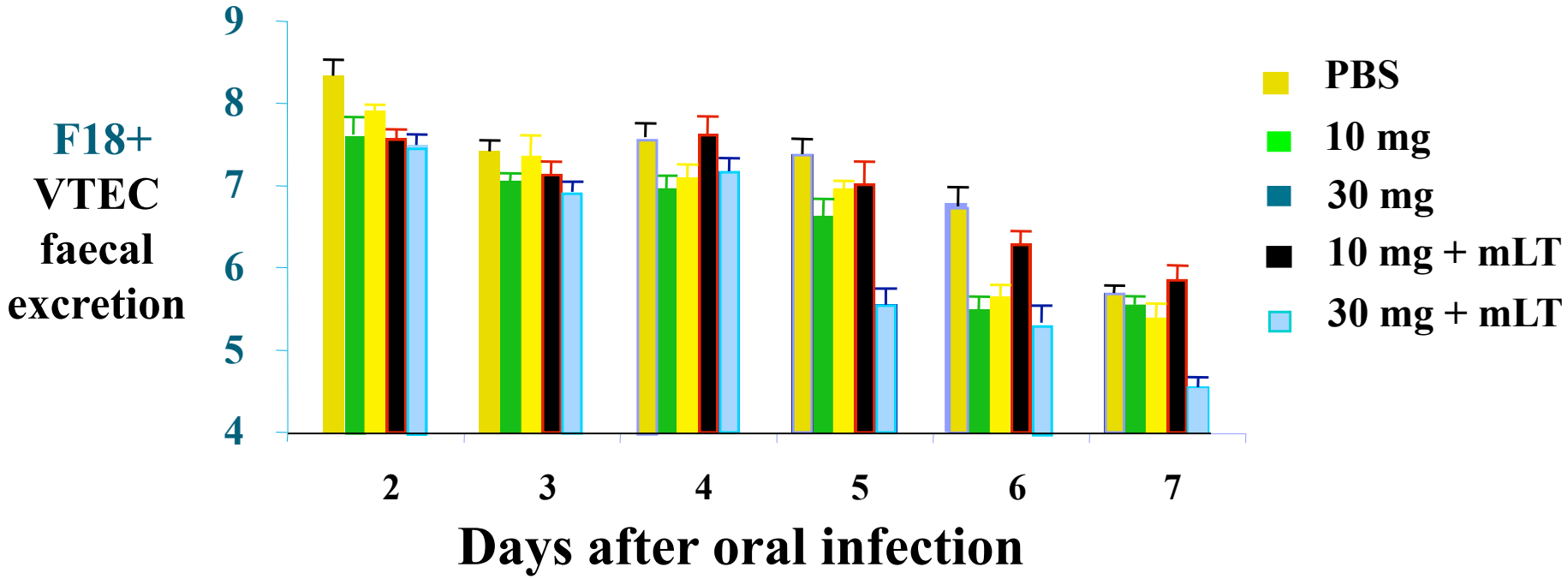
2. **Infection challenge** with
F18+ VTEC
or F4+ETEC



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

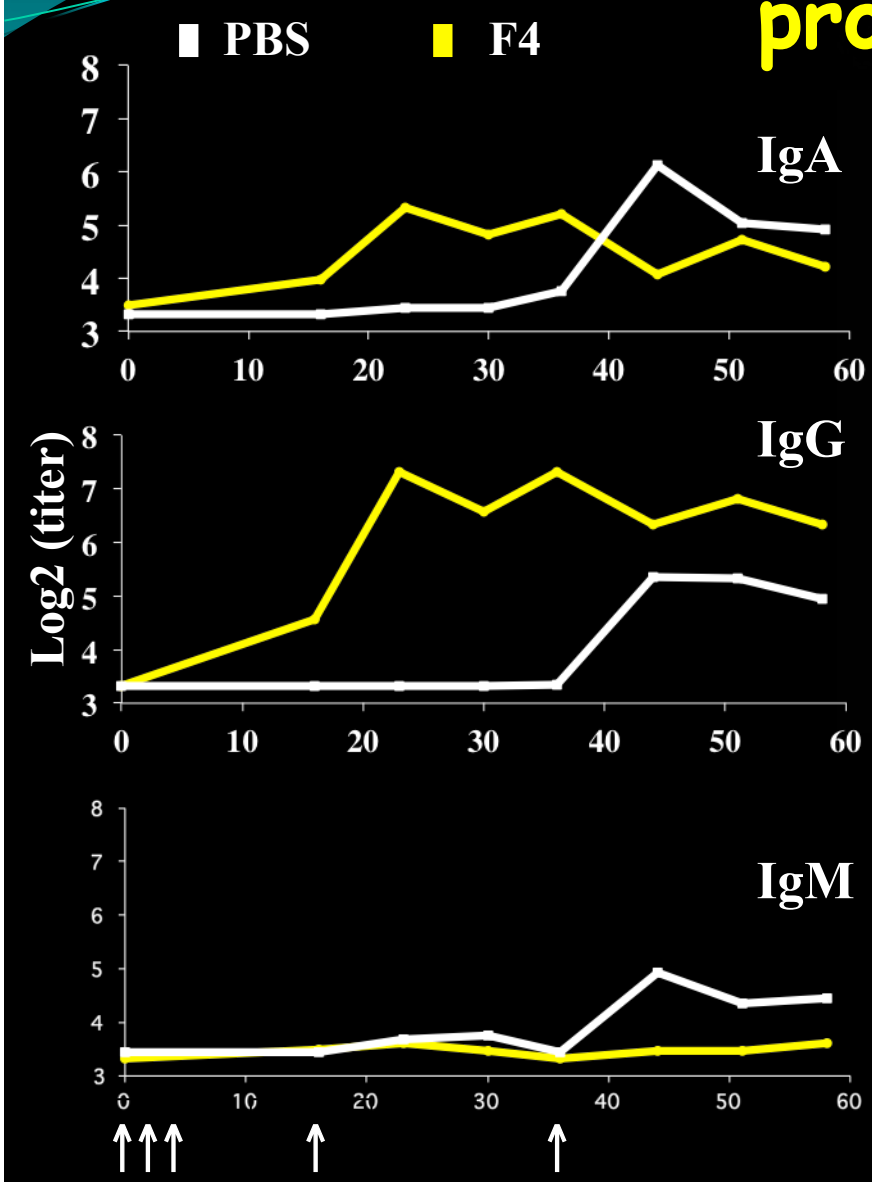
1. **Oral Immunisation**
with purified F18/F4
(and dissolved in PBS)

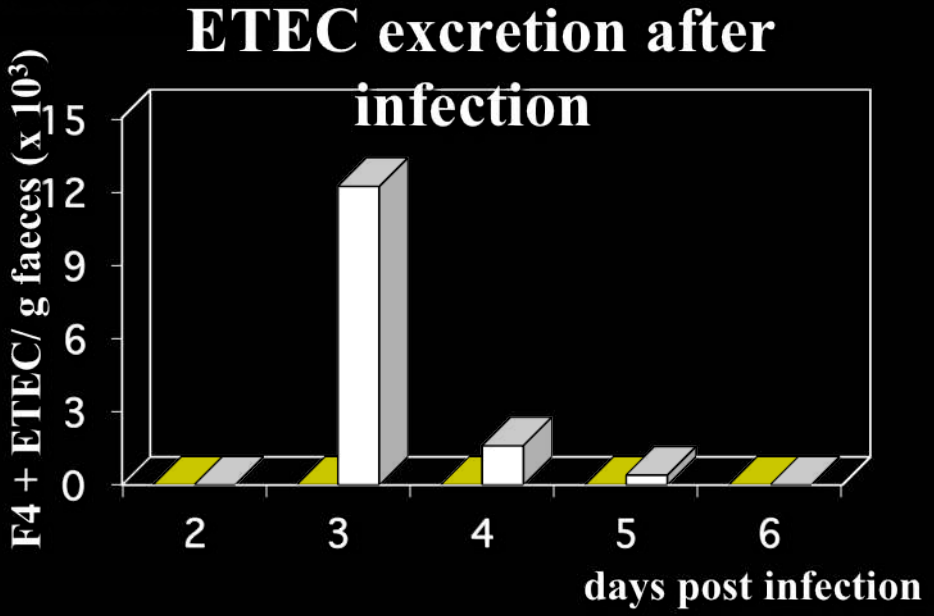
Oral vaccination with F18 fimbriae does not protect



Verdonck et al., Vet Imm Immunopath. 2007

The mucosal response against F4 is protective





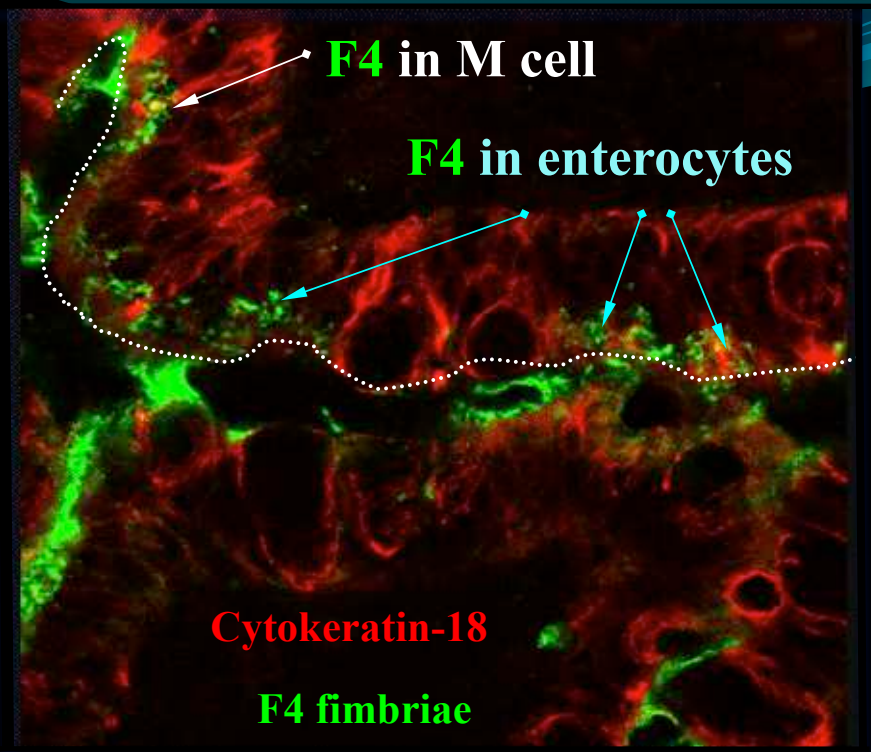
=> Oral F4 induces protective mucosal response !

Van den Broeck et al., 1999. Infect Imm

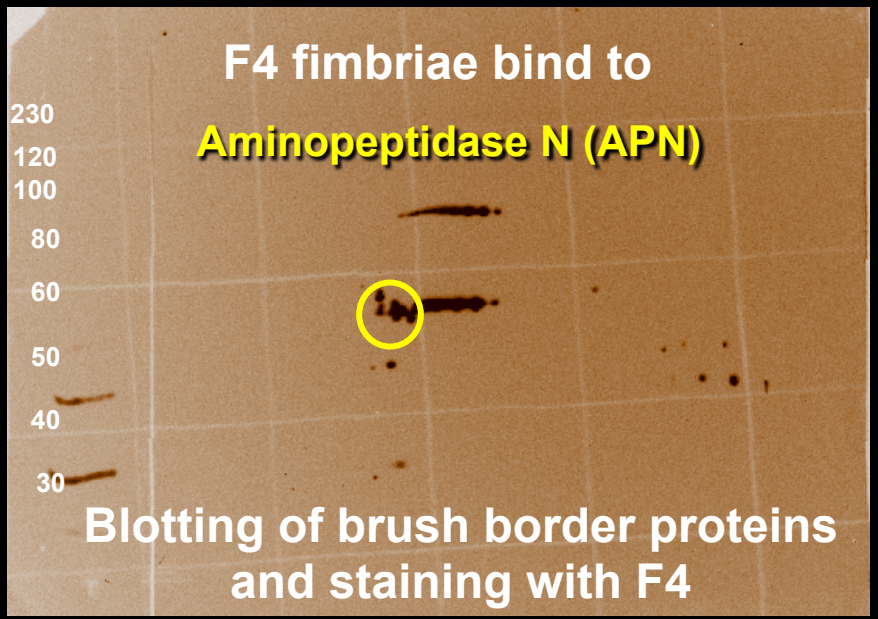
Binding and uptake of F4 fimbriae

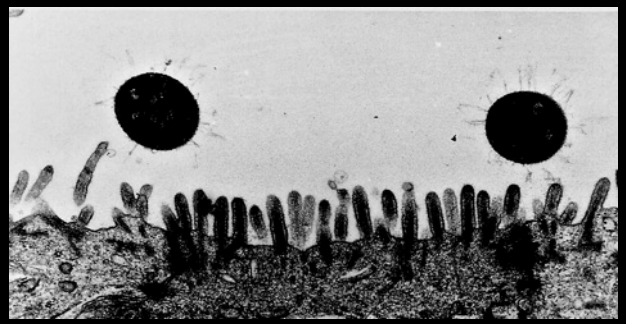
Ligated loops injected with F4

Snoeck et al., 2008. Vet Imm Immunopath.



Snoeck et al., 2008. Vet. Imm. Immunopathol



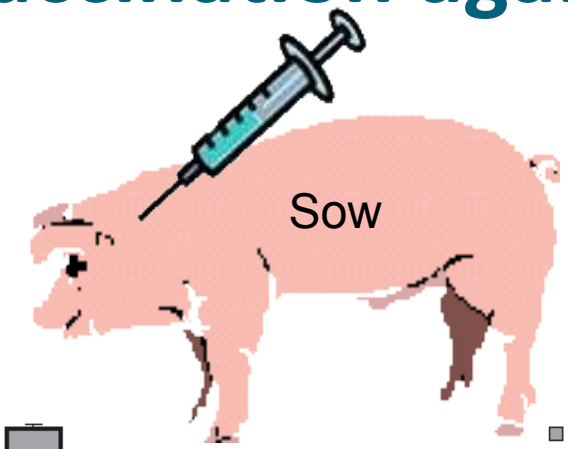


Melkebeek et al., 2012. Mucosal Immunology

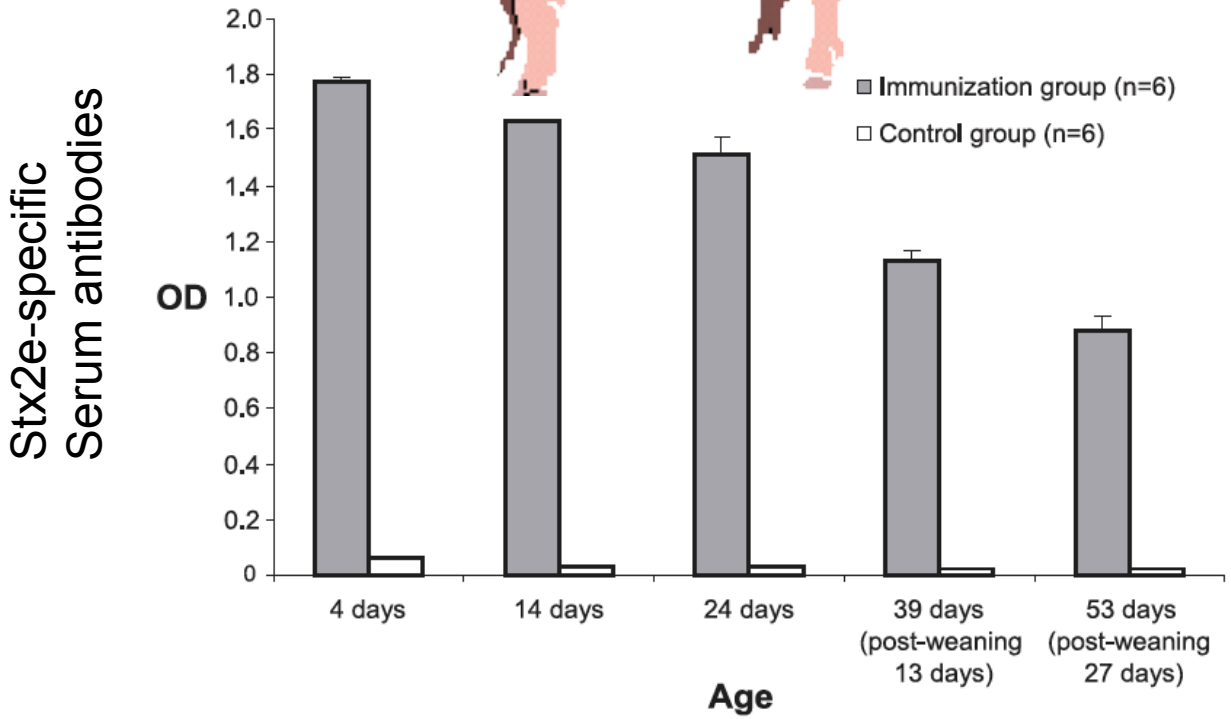
Vaccination against the toxine

Oanh et al., 2011. Inf. Imm.

Stx2e toxoid



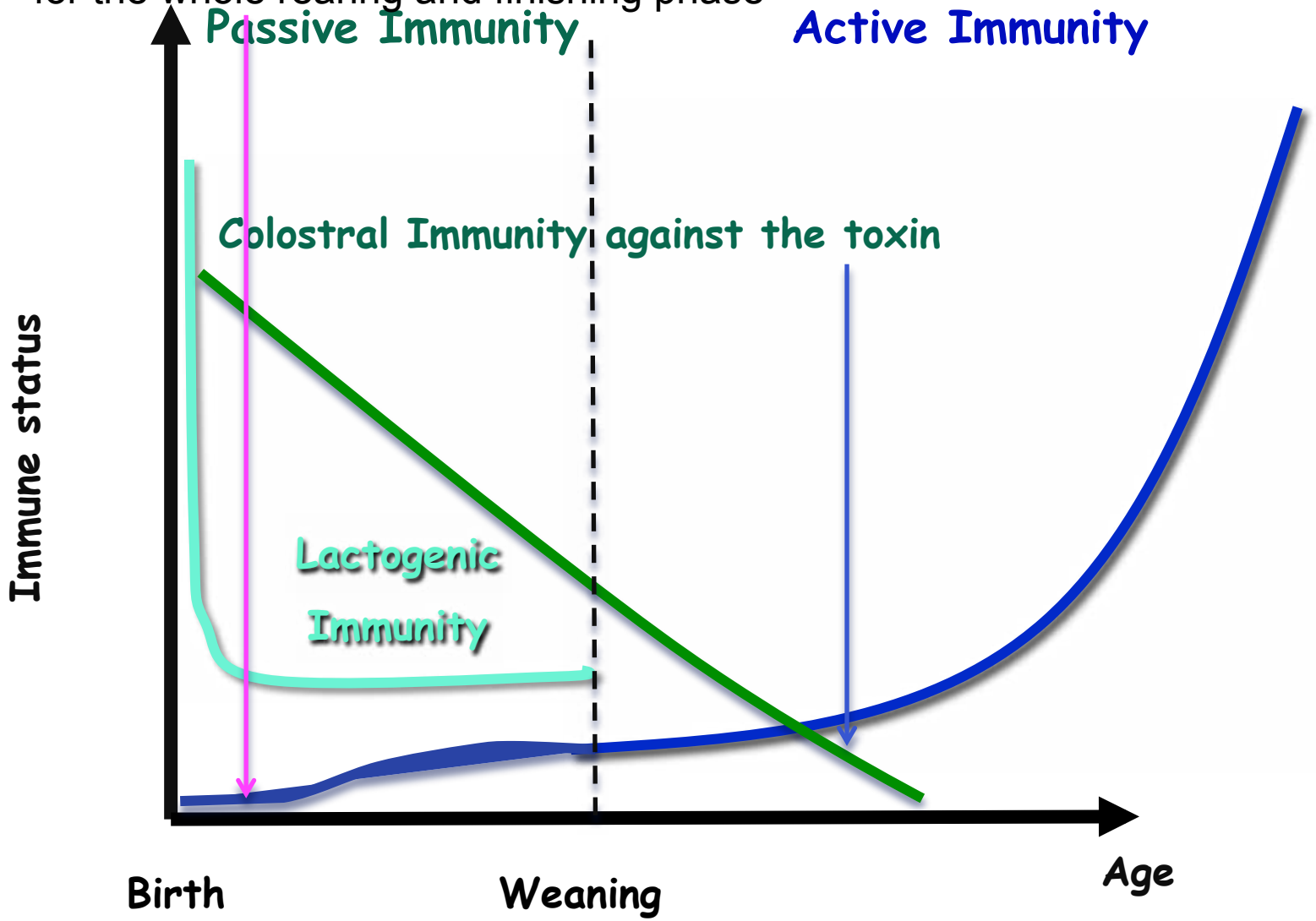
Anti-verotoxine II antibodies in colostrum



Protection of piglets against the oedema disease symptoms, but bacteria remains excreted in the faeces and is spread in the environment

German vaccine manufacturer IDT
Biologika: Ecoporc Shiga (Swiss)

Immunity develops 21 days later and lasts for the whole rearing and finishing phase



Conclusions

- Oral immunisation remains a challenge
- Parenteral vaccination against the toxins is an interesting route with a lot of potential
- Inhibition of colonisation with sugars is examined in the experimental infection models
- The genetic selection should be restarted re

Acknowledgements

Former PhD students)

Prof. Dr. Wim Van den Broeck
dr. Frank Verdonck (Ablynx)
dr. Petra Tiels (VIB-UGent)
Karien van Gog (practice)
dr. Veerle Snoeck (Ablynx)
dr. Kristien Rasschaert (B&D)

Lab of Immunology (UGent)

Prof dr. Bruno Goddeeris (Lab Immuno/KULeu
Prof. Dr. H. Favoreel
dr. Annelies Coddens
dr. Vesna Melkebeek
dr. Bert Devriendt
Lic. Philippe Bellot

Lab of Pharmaceutical Technology (UGent)

Prof. Dr. Jean-Paul Remon
Prof. Dr. Bruno De Geest
dr. Katrien Vandamme
dr. Nathalie Huyghebaert
dr. An Vermeire

Vrije Universiteit Brussel (VUB)

Prof. Dr. Henri Degreve and Han Remaut

Provaxs (Ugent)

dr. Sven Arnouts

Financial support

Faculty of Veterinary Medicine

UNIVERSITEIT GENT

PROVAXS

Lab of Immunology and biotechnology of the (Ugent)
Prof. dr. D. Vanrompay

Lab of Pharmaceutical Biotechnology (Ugent)
Prof dr. Dieter Deforce
dr. Kelly Tilleman<

University of Gothenburg, Sweden
Prof. dr Susann Teneberg

federale overheidsdienst
VOLKSGEZONDHEID, VEILIGHEID VAN DE VOEDSELKETEN EN LEEFMILIEU

iwt

FWO VLAANDEREN
Fonds Wetenschappelijk Onderzoek
Research Foundation – Flanders

UNIVERSITEIT GENT