UNIVERSITEIT GENT

Labo Immuno

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

Receptor analogues as strategy to prevent *E. coli* infection

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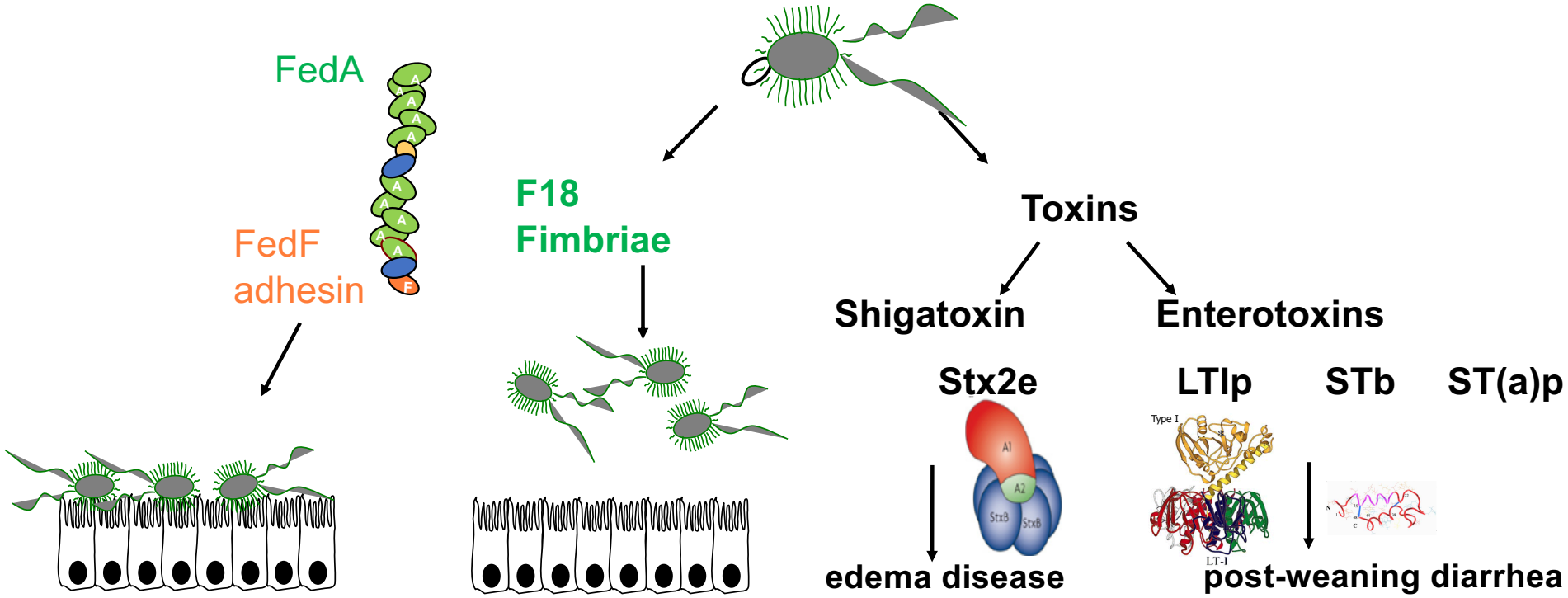
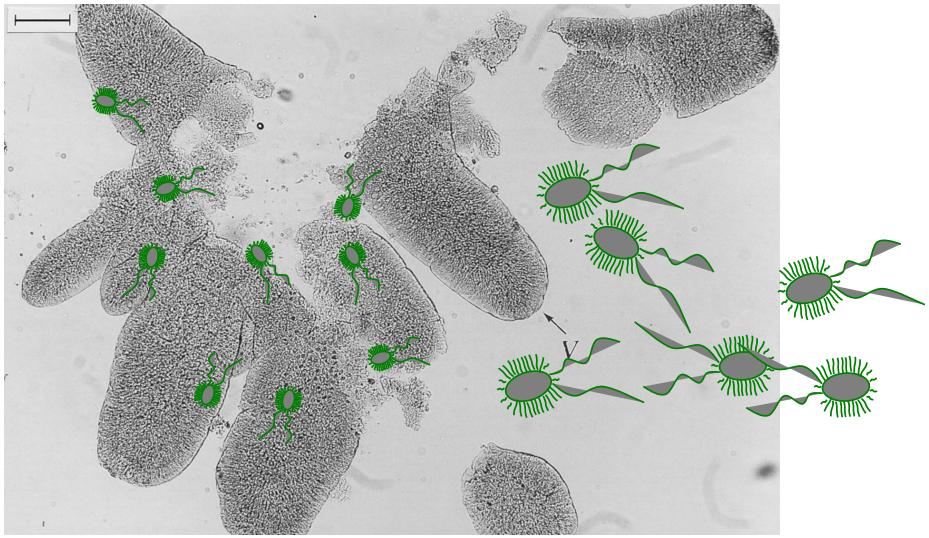
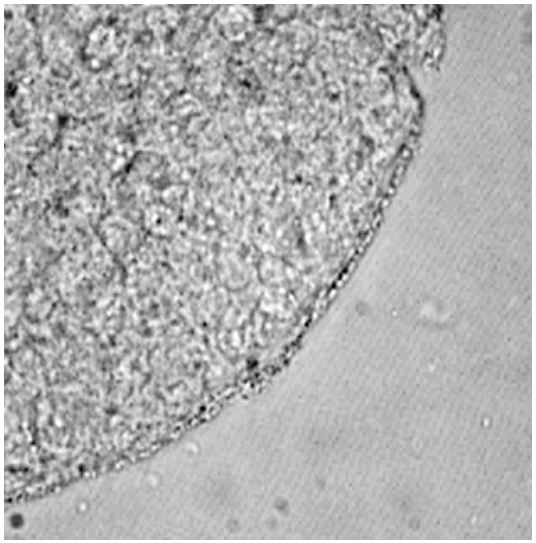
F18+ *Escherichia coli*

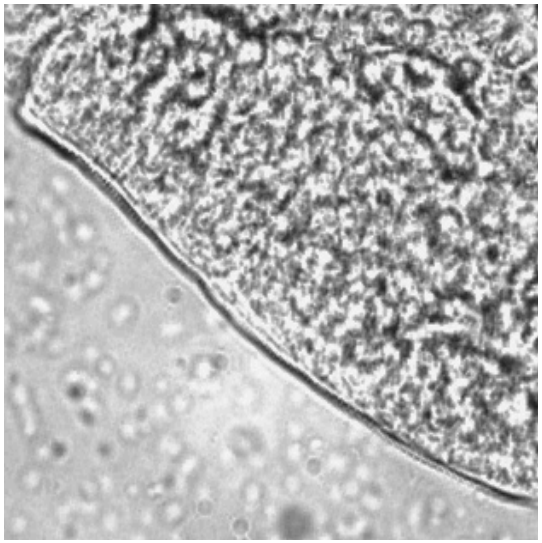


In vitro villous adhesion assay





F18R positive piglets



F18R negative piglets

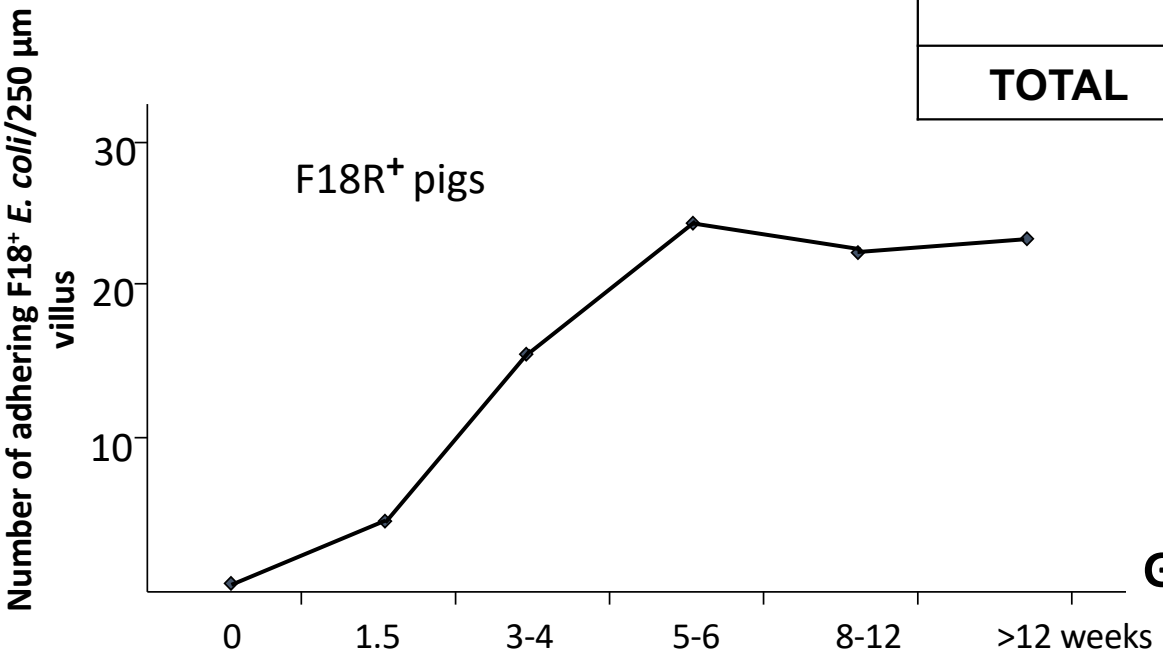
Cox and Houvenaghel, 1993. Vet. Microbiol.

F18R correlated with a point mutation in *FUT1*

Chromosome 6

FUT1 mutation ➔Not 100% absolute

		FUT1-genotype			Total
		GG	AG	AA	
		SS	SR	RR	
ADHESION	STRONG	35	23	0	58
	WEAK	8	6	1	15
	NEGATIVE	1	2	7	10
TOTAL		44	31	8	83



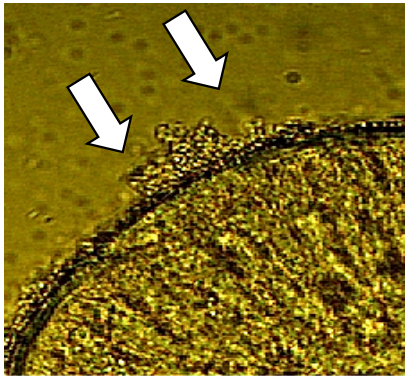
F18R expression pattern increases in young pigs with age

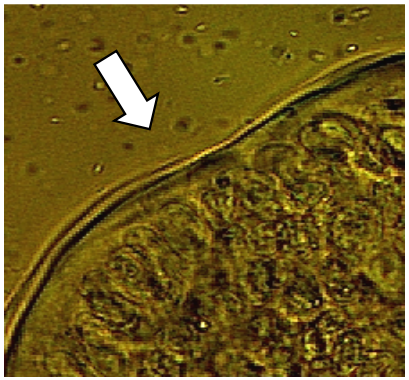
> 5-6 weeks old

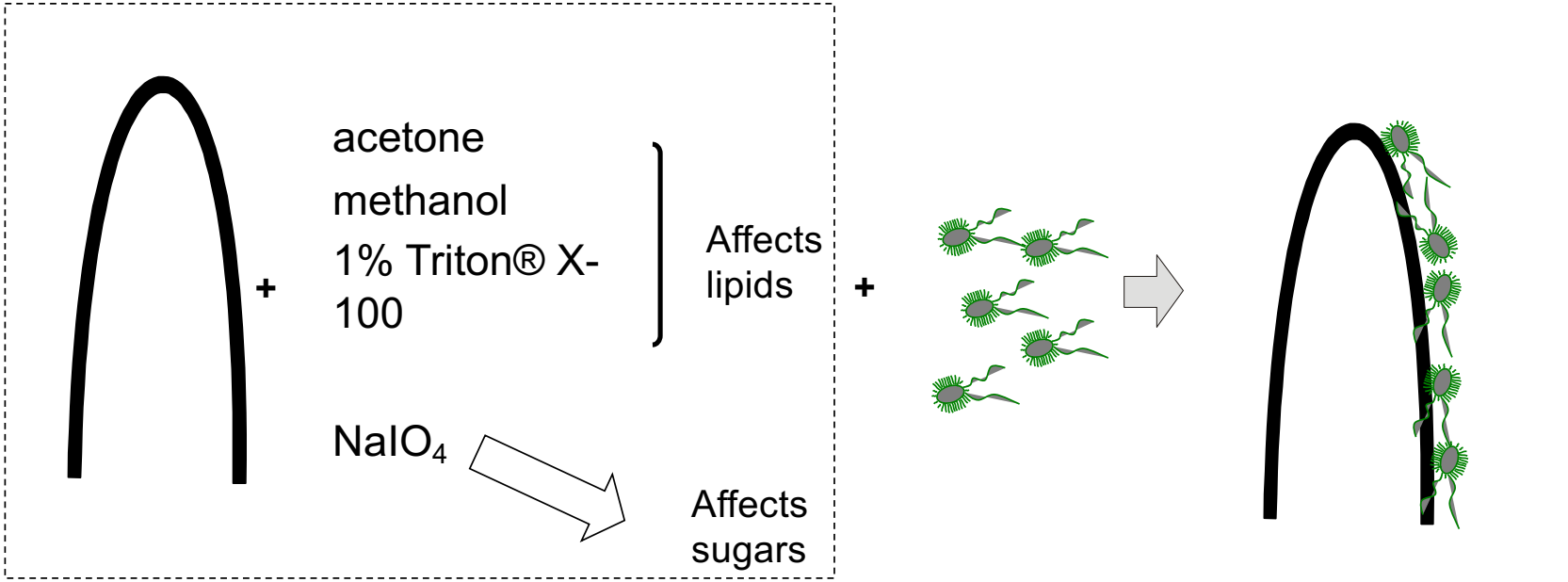
Genotype has to be confirmed in phenotype

Coddens et al., 2007. Vet. Microbiol.

The physiochemical properties of the F18R



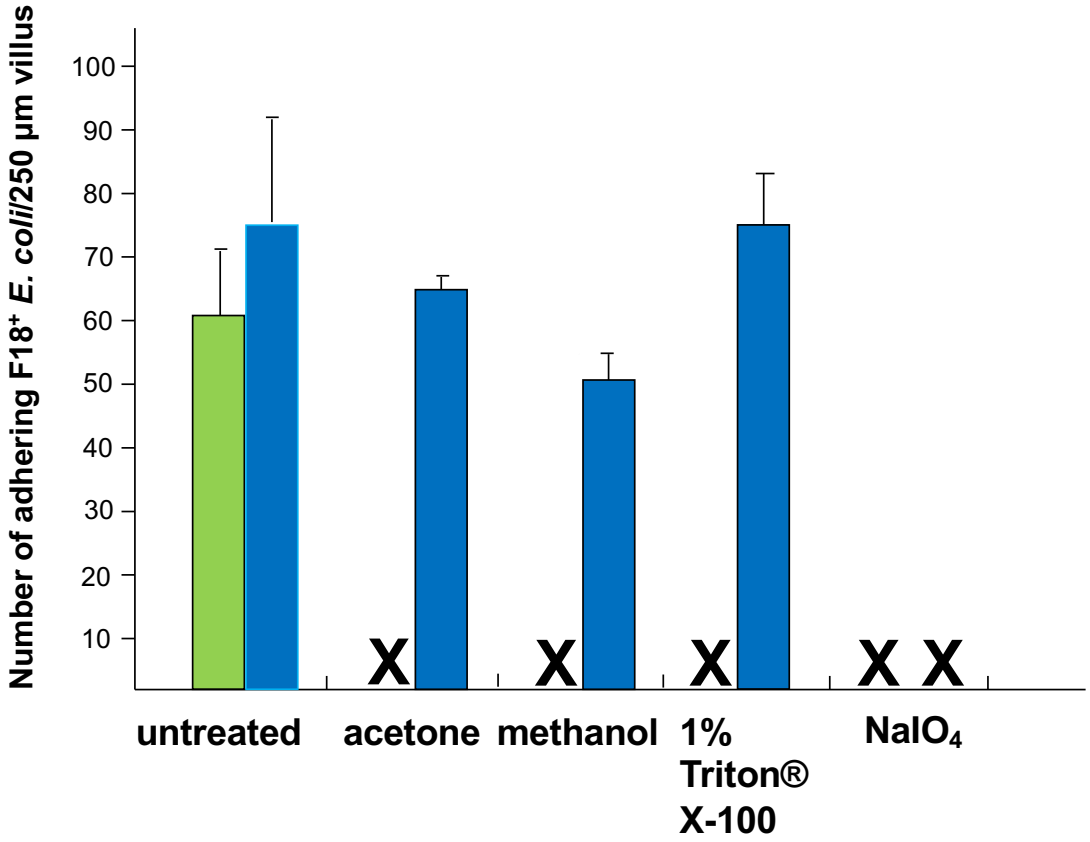




Pre-incubation of F18R⁺ villi + **F18⁺ *E. coli*** + **adhesion ?**

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

The physiochemical properties of the F18R



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

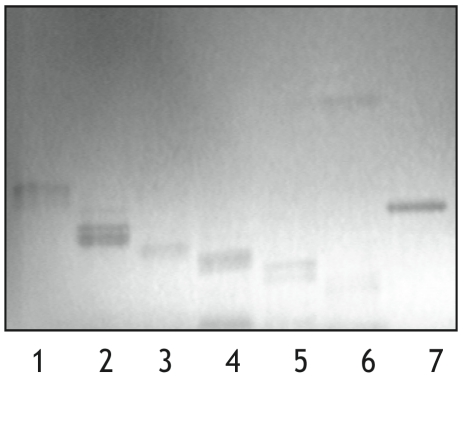
F4ac$^+$ *E. coli* (n = 2)

=> The F18R is a **glycolipid**

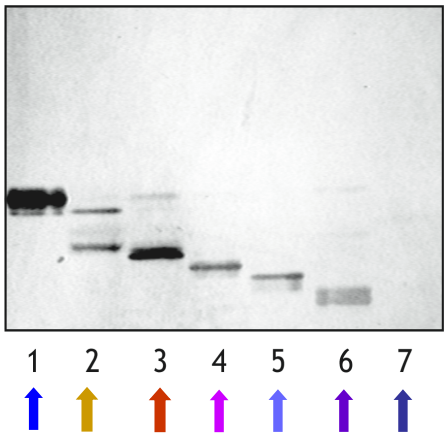
Coddens et al., 2009. J. Biol. Chem.

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

A. Chemical detection



B. *E. coli* HB101(pIH120) **+ FedF**



Mass spectrometry

Proton NMR spectroscopy

Susann Teneberg, Institute of Biomedicine, University of Ghotenburg

Blood group O pig =>	H5 type 1	Fucα2Galβ3GlcNAcβ3Galβ4Glcβ1Cer
Blood group A pig =>	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	

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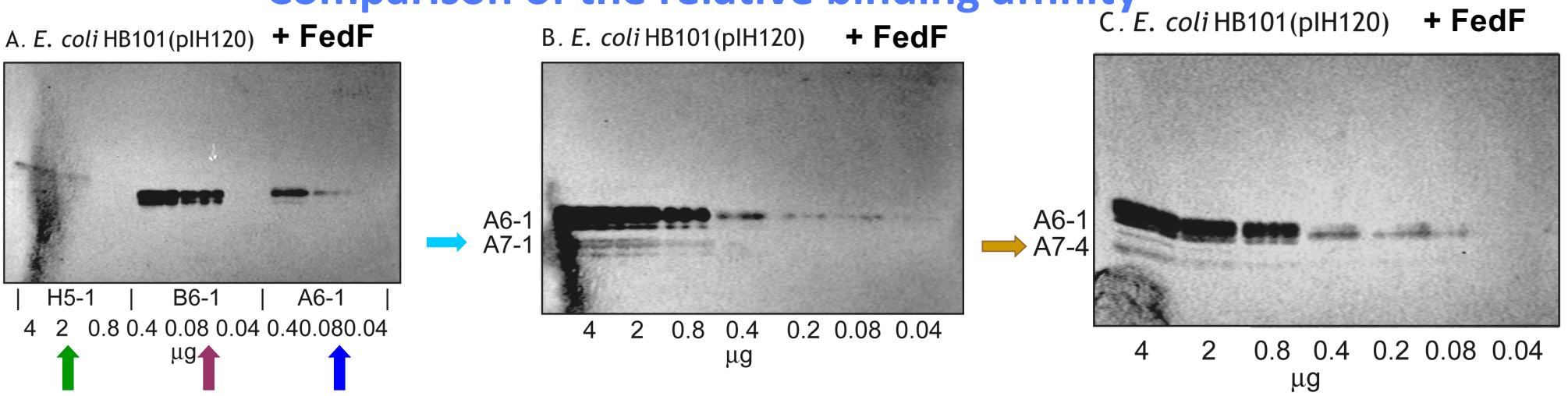
Coddens et al., 2009. J. Biol. Chem.

Comparison of the relative binding affinity



H5 type 1 (2 µg) Fucα2Galβ3GlcNAcβ3Galβ4Glcβ1Cer

<< **B6 type 1 (0.08 µg)** Galα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer

<< **A6 type 1 (0.08 µg)** GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer

V

V

A7 type 1 (0.8 µg) GalNAcα3(Fucα2)Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer

A7 type 4 (0.8 µg) GalNAcα3(Fucα2)Galβ3GalNAcβ3Galα4Galβ4Glcβ1Cer

=> **Terminal α3-linked galactose or GalNAc contributes significantly to the interaction**

=> **Fuca4 on GlcNAc constitutes a sterical hindrance for interaction with F18⁺ *E. coli***

=> **Internal GlcNAc contributes significantly to the interaction**

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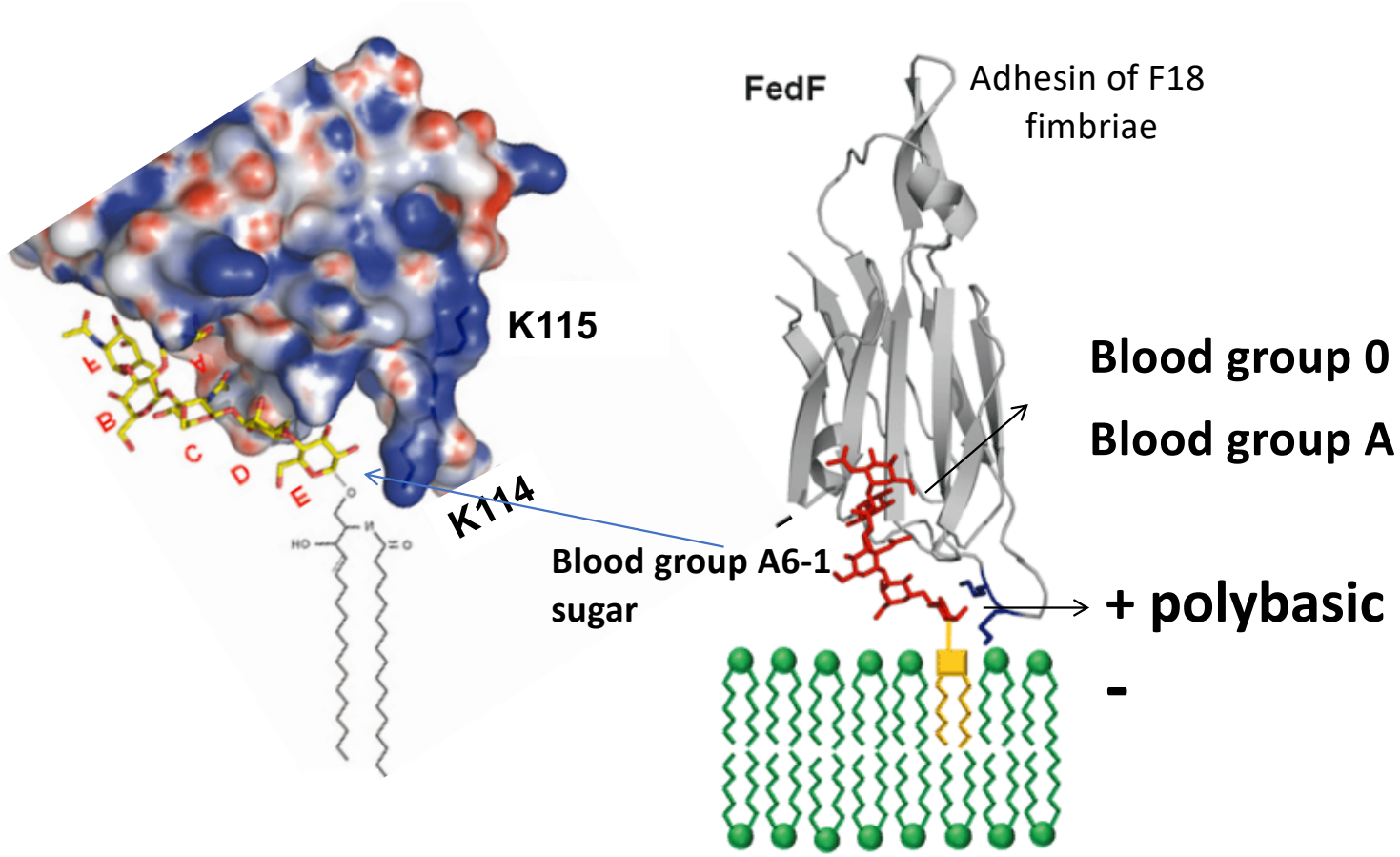
Coddens et al., 2009. J. Biol. Chem.

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



1/ interaction of FedF with blood group sugars (very specific)
2/ interaction of FedF (K114/K115) with acidic lipids in membrane (less specific)

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

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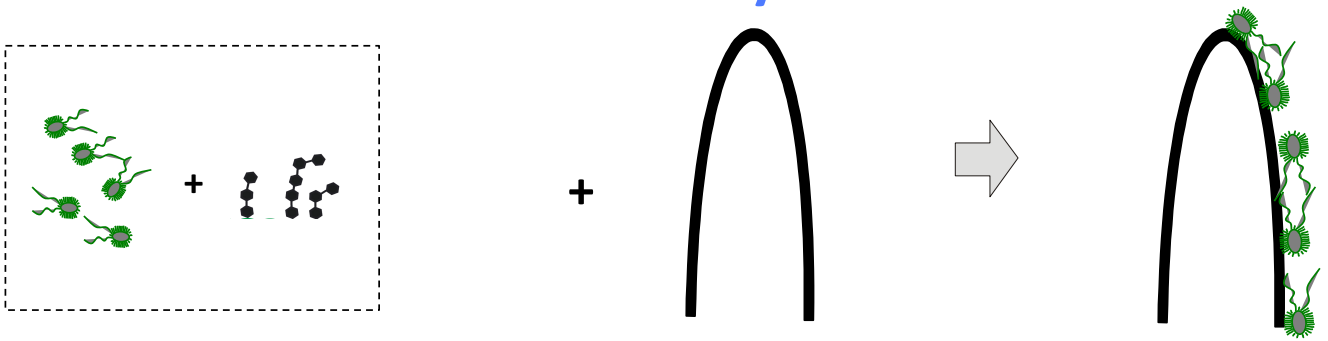
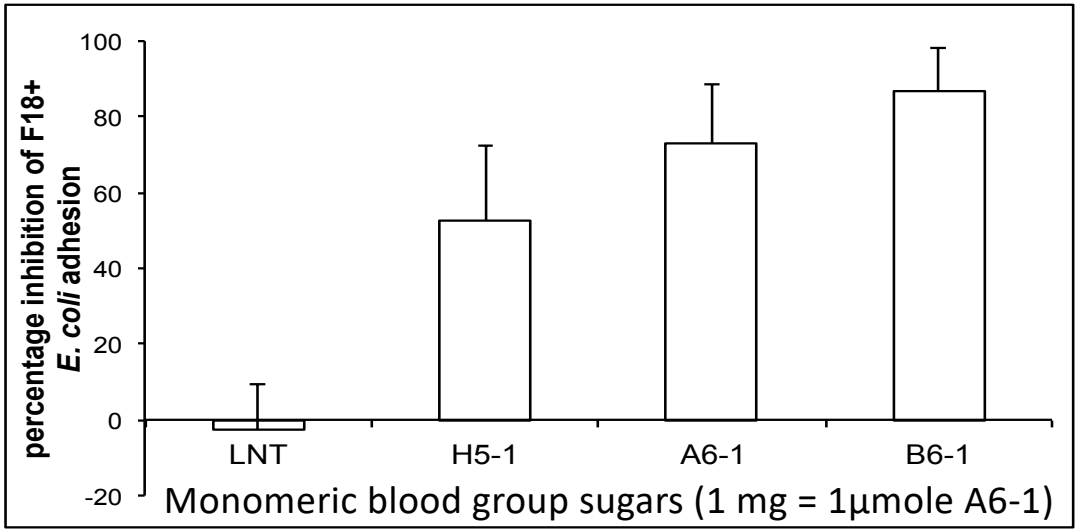
In vitro villus adhesion to study interaction of *E. coli* with the villi and inhibition by molecules

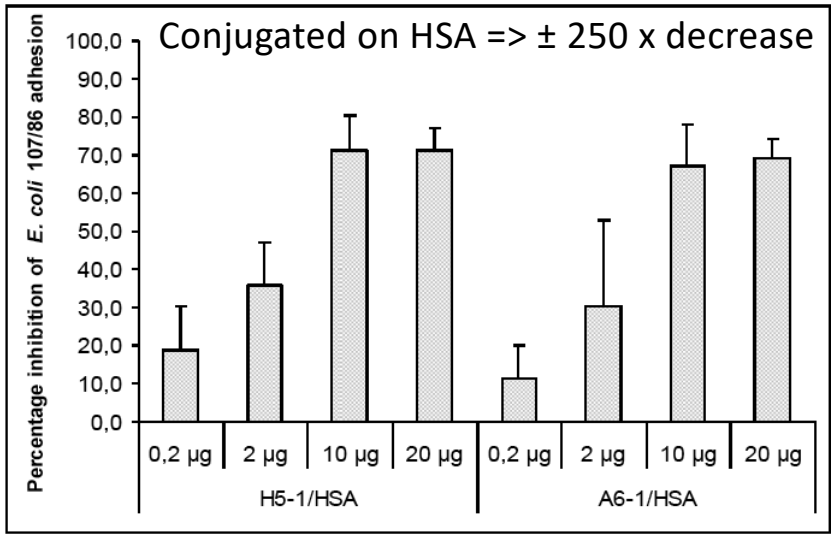


Pre-incubation of bacteria with H5-1, A6-1, B6-1 sugars

Are added to small intestinal villi of F18R+ piglets

Percent inhibition of adhesion is calculated ?





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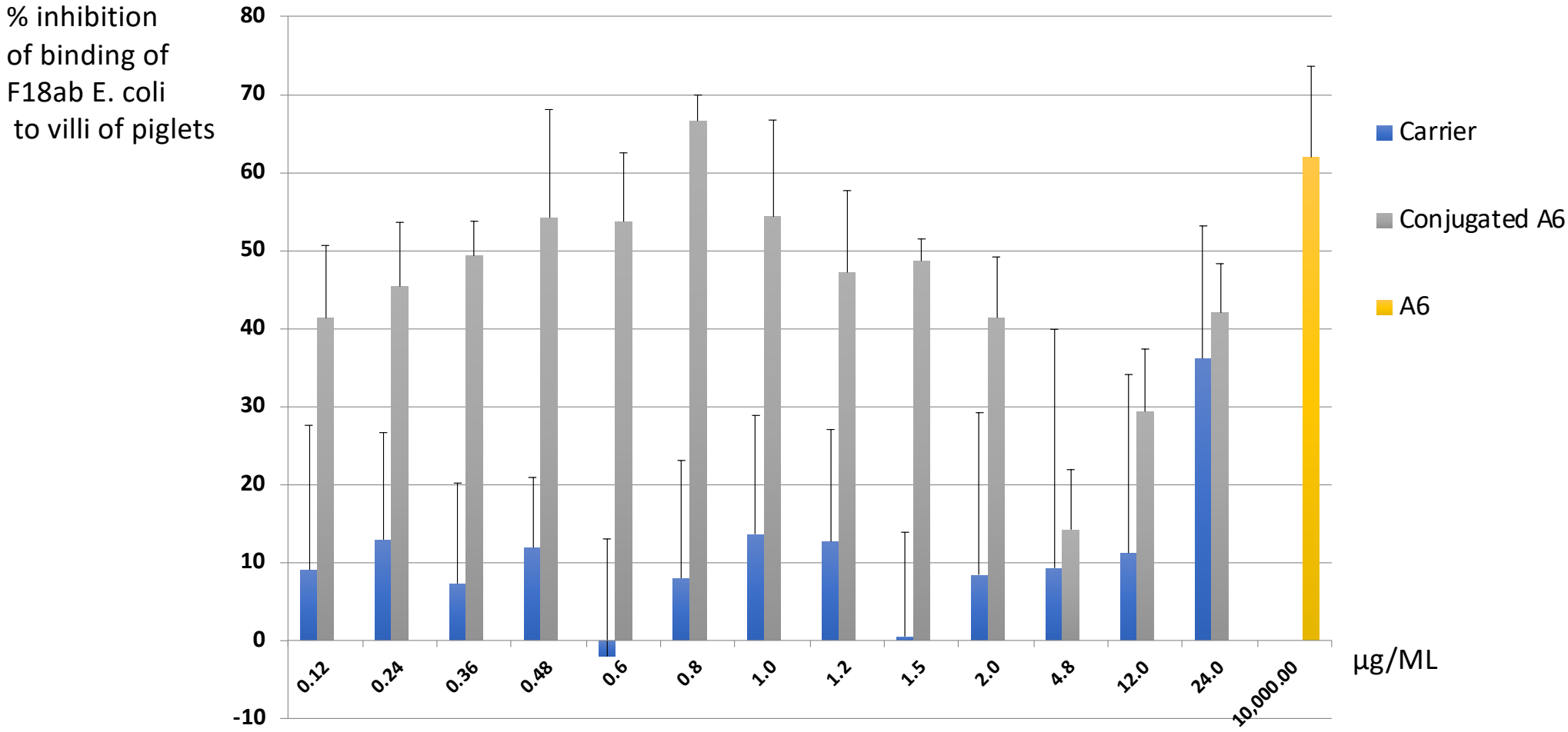
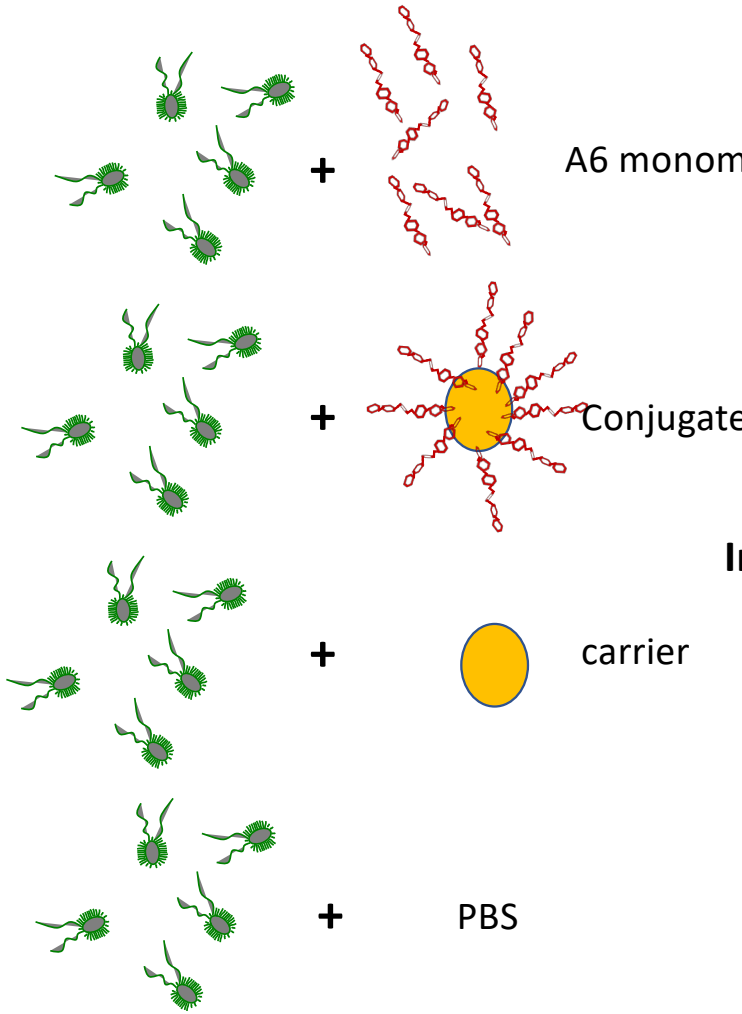
Coddens, Cox, Teneberg, 2014. Inhibitors of F18+ *E. coli* binding, patent

Capacity of conjugated A6 to inhibit *in vitro* binding of F18 *E. coli*

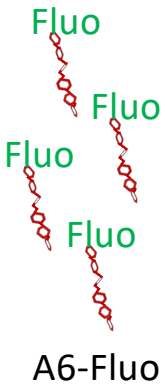


The inhibition by the conjugate is more than 10,000 times higher than for the monomer

The inhibition is specific

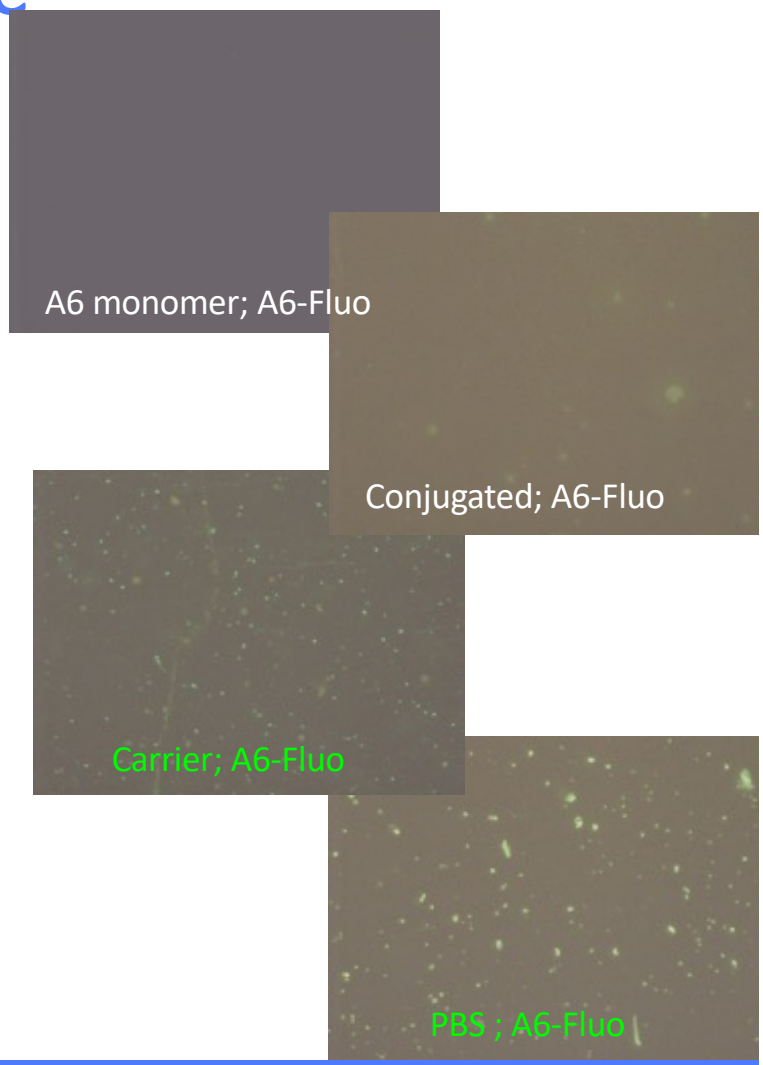


Incubate



A6-Fluo

Incubate



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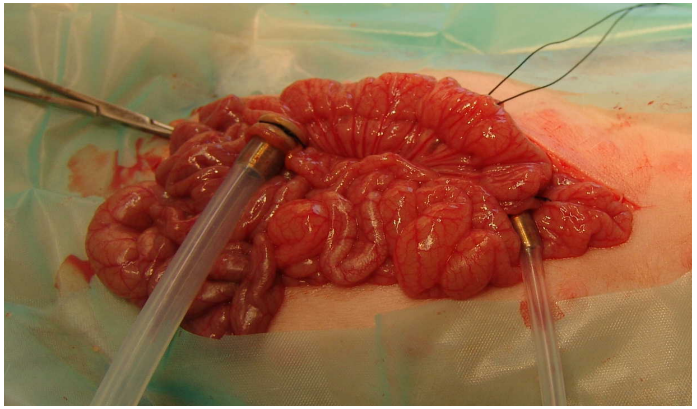
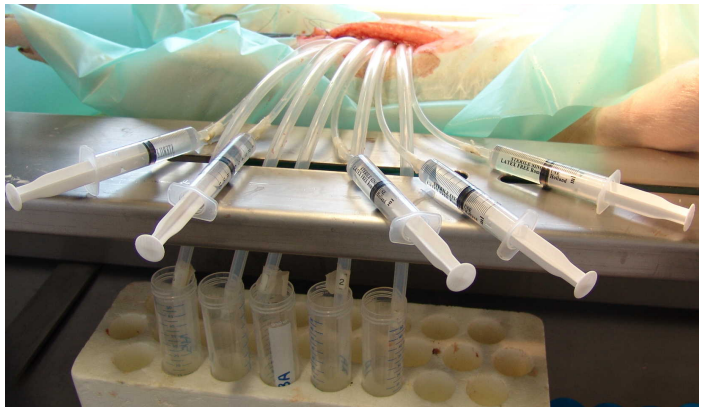
Small intestinal segment perfusion (SISP) model

- Weaned piglets
- Inhalation anesthesia
- 6 segments in jejunum
  - Start ±2 m from stomach
  - 20 cm long
  - Cranial inflow tube (Ø 3 mm) => sugar and F18ac$^+$ STa$^+$ STb$^+$ *E. coli*
  - **Caudal outflow tube (Ø 5** mm)=> Fluid collected at outlet

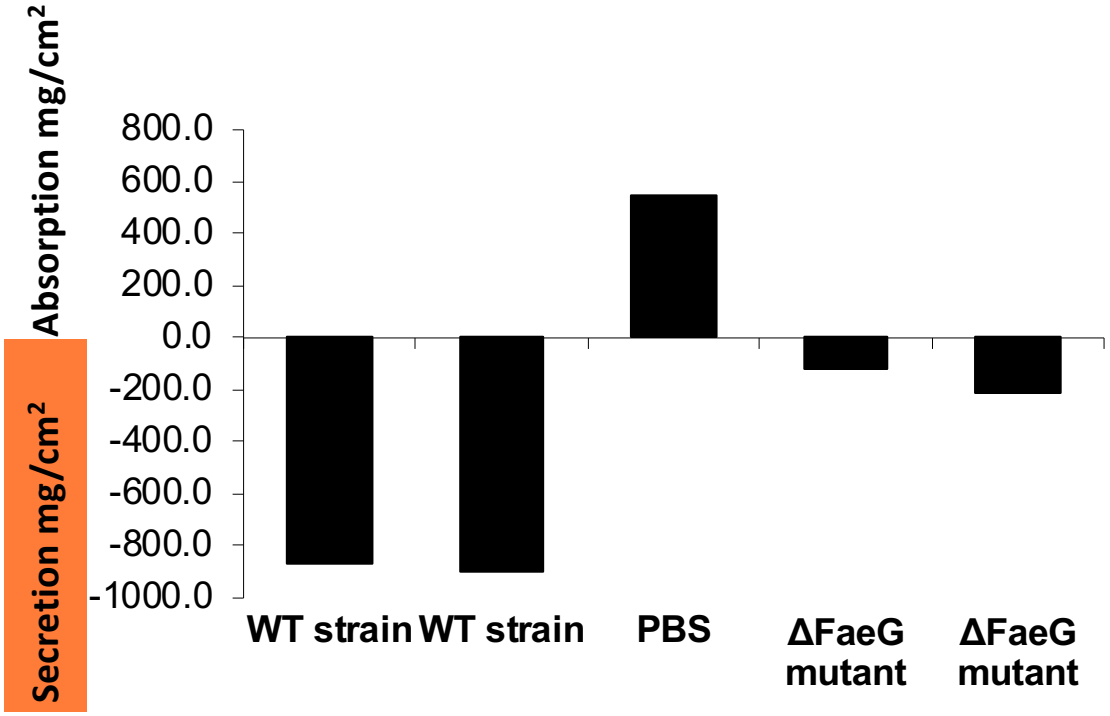




Colonization is essential for diarrhoea in the SISP model

F4R$^+$ pig, **8h** perfusion

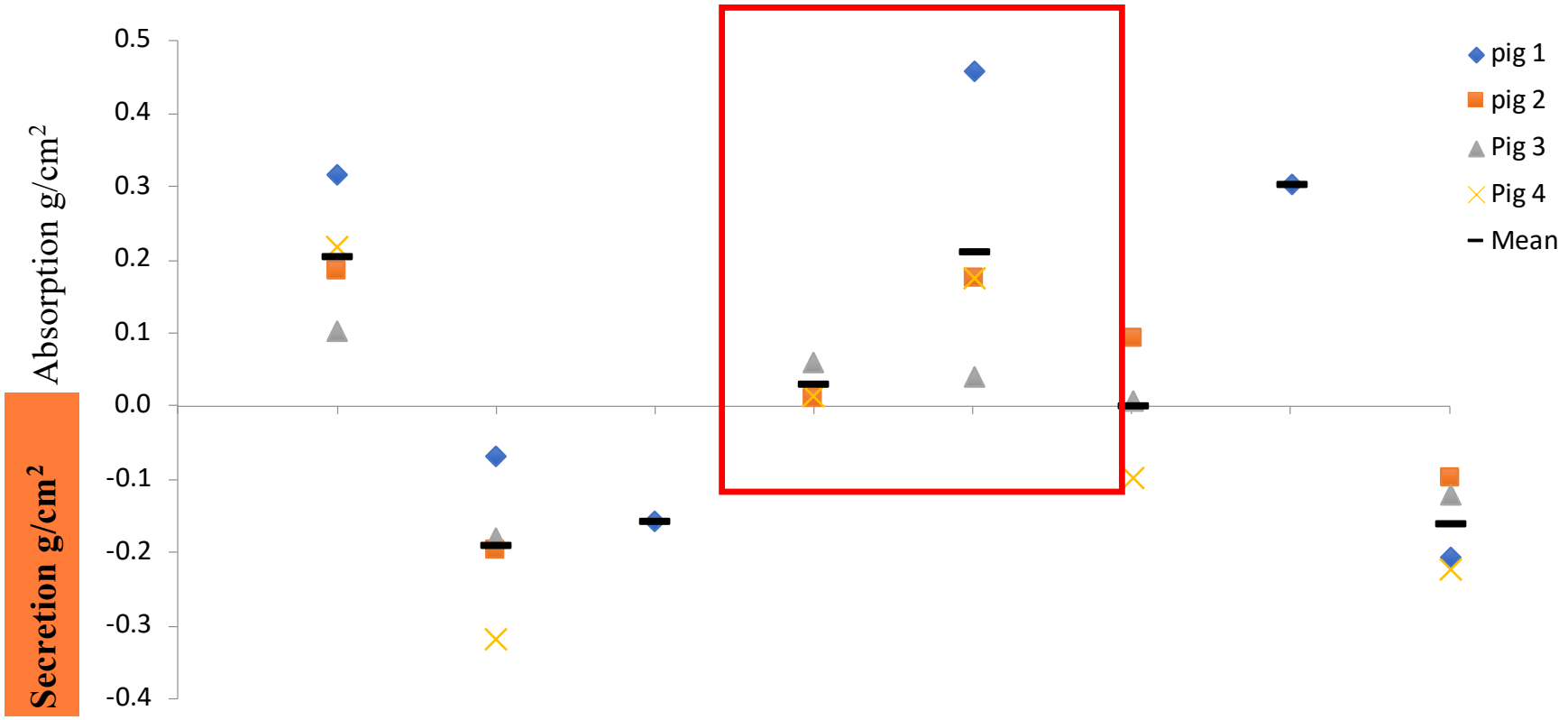
- Compare wild type F4ac$^+$LT$^+$STa$^+$STb$^+$ *E. coli*
- with mutant strain lacking F4 (deletion of FaeG subunit) (**F4ac$^-$**LT$^+$Sta$^+$STb$^+$)



F4 mediated adhesion results in severe fluid loss

Loos and Cox, UGent, 2013, unpublished results

Conjugated A6 can decrease net secretion of an F18ac+ *E. coli* strain producing STa and STb enterotoxins in a small intestinal segment perfusion assay

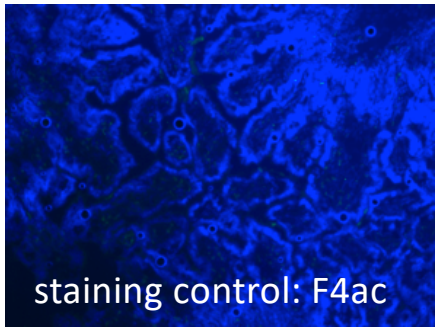


Bacteria	F4ac+toxin-	F18ac+	F18ac+	F18ac+	F18ac+	F18ac+	F18ac+	F18ac+
Conjugaat in µg/ml	2.6	0	1.04	2.6	10.4	41.4	104	Carrier only 41.6

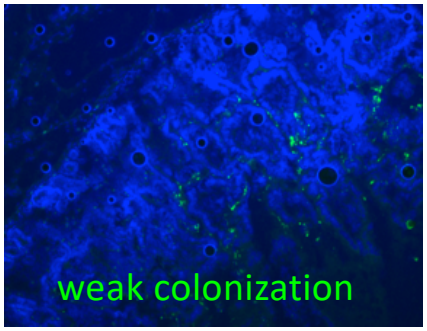
Inhibition shown on cryosections

Cryosections stained with

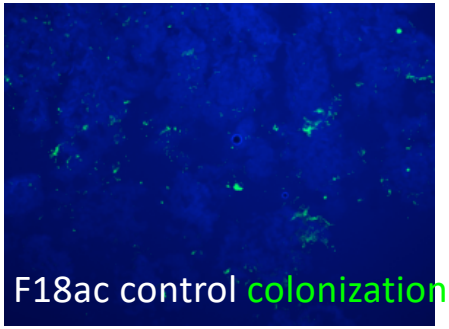
- Mab anti-FedA antibodies and anti-mouse FITC (green fluorescence)
- Nuclei with Hoechst (blue fluorescence).



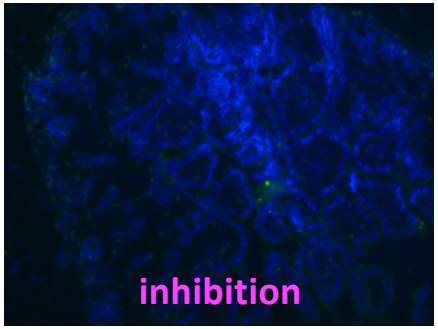
F4ac *E. coli* STb- LT- 2.6 ug/ml conjugate



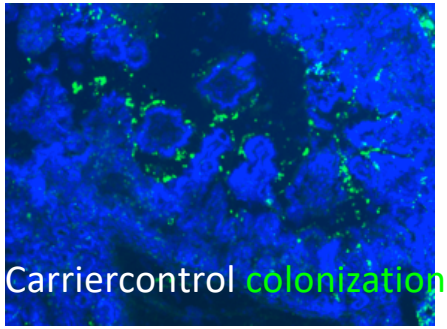
F18ac+ *E. coli* 1.04 ug/ml Conjugate



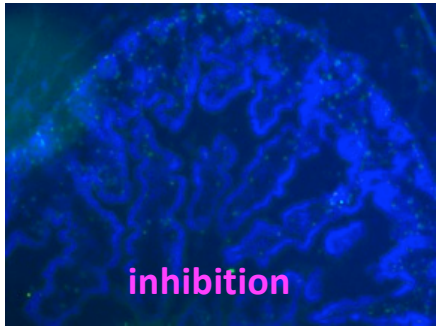
F18ac+ *E. coli* PBS



F18ac+ *E. coli* 10.4 ug/ml Conjugate



F18ac+ *E. coli* 41.6 ug/ml carrier



F18ac+ *E. coli* 104 ug/ml Conjugate

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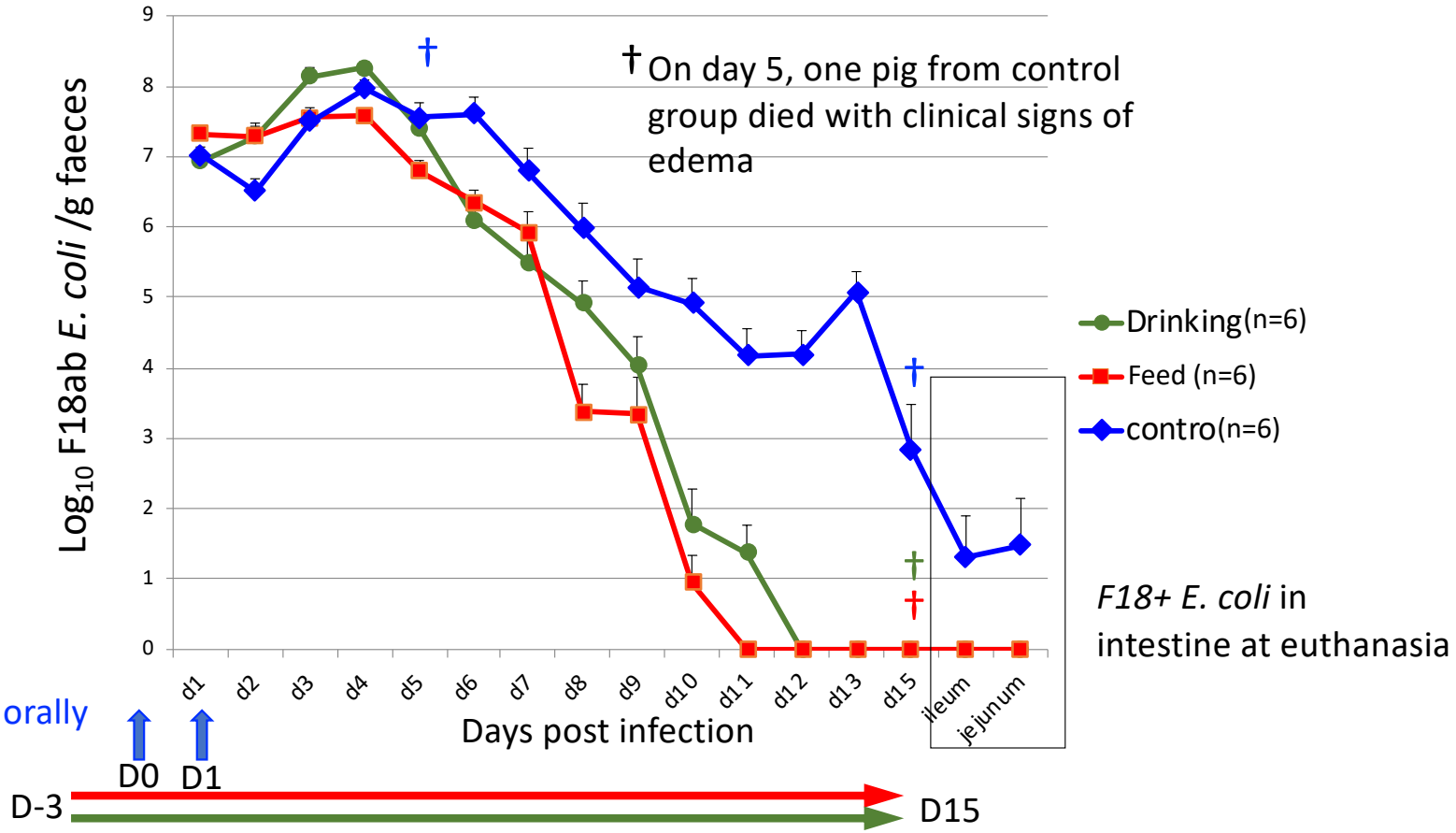
Survival and F18ab+ Stx2e *E. coli* shedding for piglets supplemented with conjugate



All pigs stopped excreting F18⁺ *E. coli* in feces from day 15, except 2 pigs from the control group. One of both pigs had the challenge strain in jejunum and ileum at euthanasia

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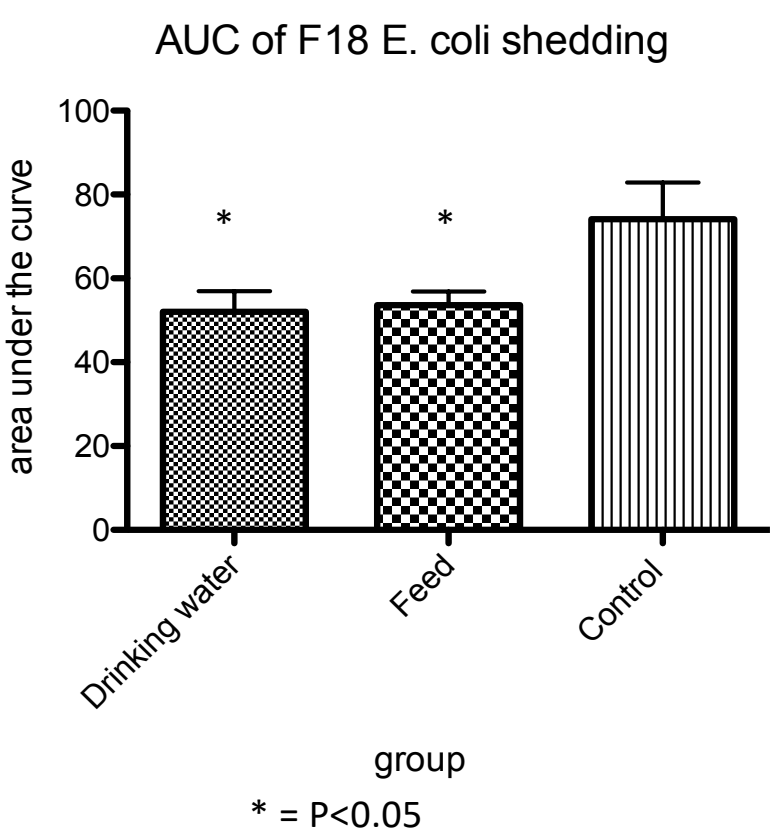
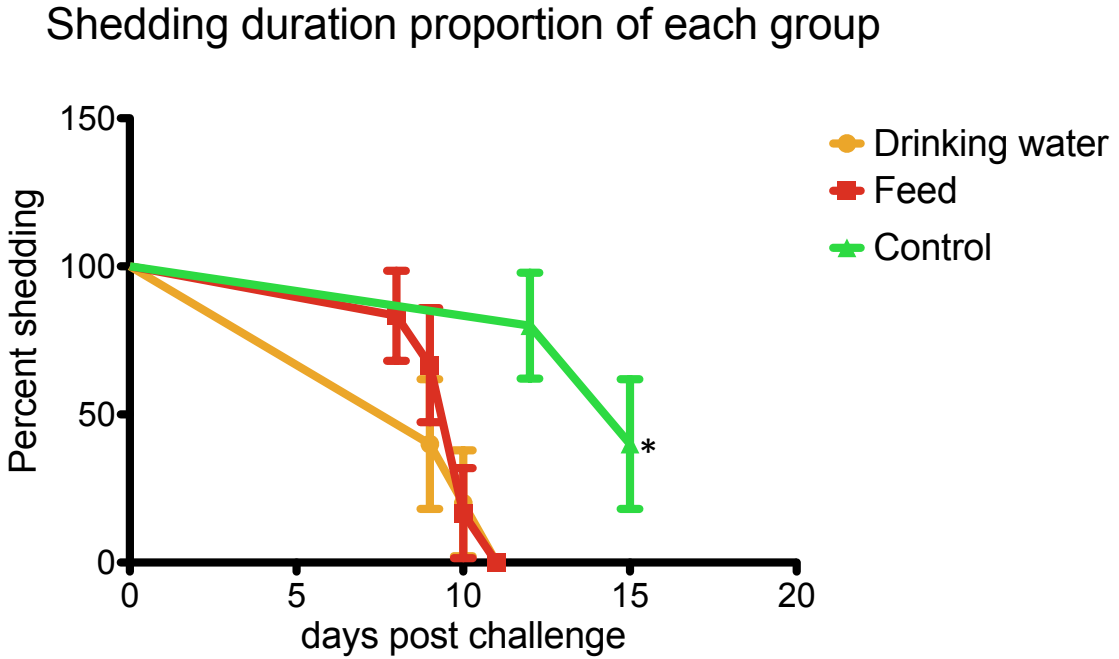
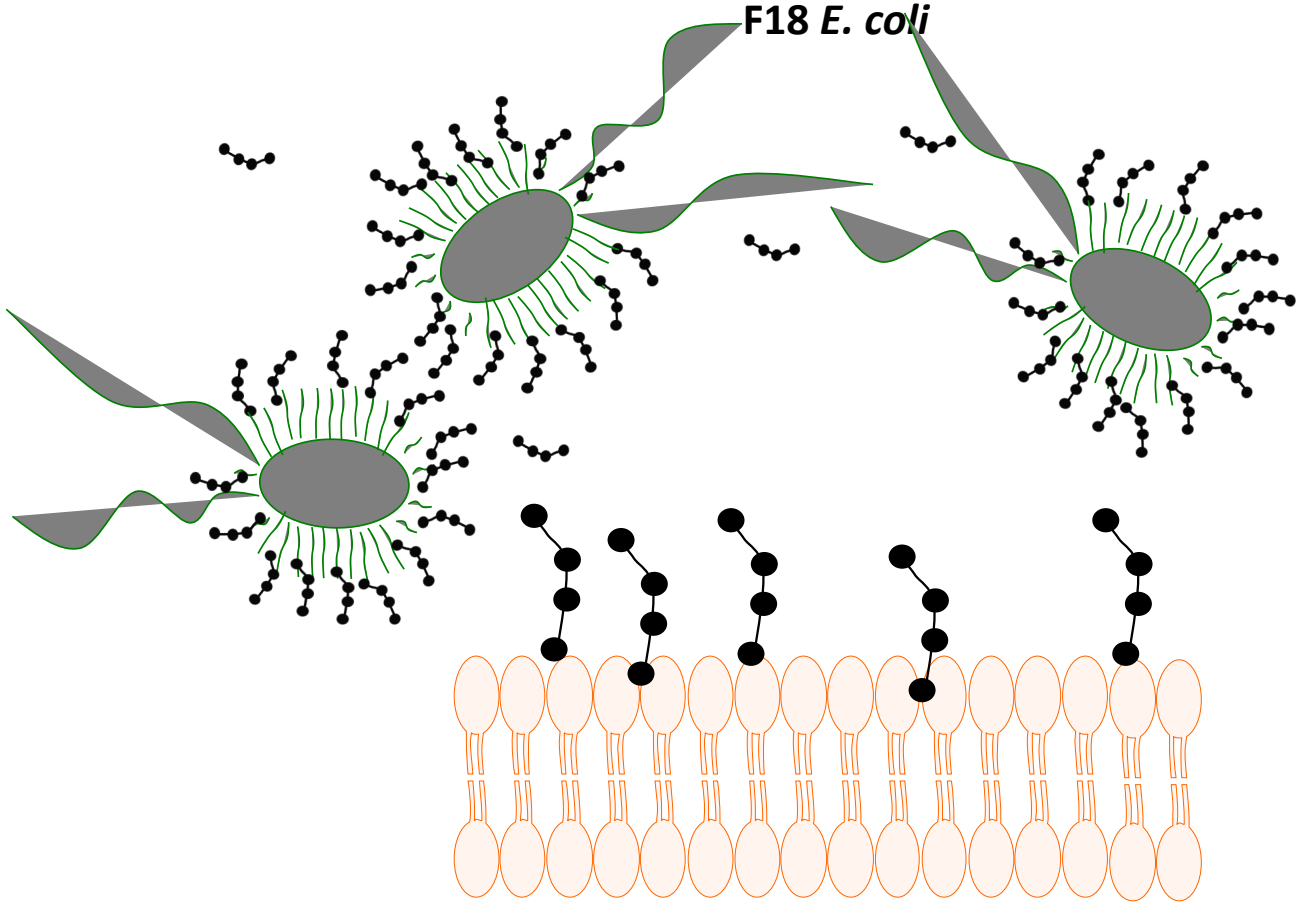


* = P<0.05



Duration of excretion is significantly shorter in both treated groups than the control group

A6-conjugate decreases colonization with F18 *E. coli*



Field studies on the efficacy of Conjugate to prevent F18^{+} *E.coli* disease in newly weaned pigs

Farm 1 with F18ab^{+} *E. coli* STa^{+}STx2e^{+}

- 4 groups of 24 F18R+ pigs (12 male and 12 female): 2 control groups (C1 and C2) and 2 treated groups (P1 and P2), with C1-P1 and C2-P2 being siblings
- Supplementation in the feed for 14 days

Farm 2 with F18ac+ *E. coli* STa+LT+

- 3 groups of 22 F18R+ pigs (11 male and 11 female): 2 control groups (C3 and C4) and 1 treated group (P3), no siblings
- Supplementation in the feed from 3 days before till 14 days after weaning

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Conclusions

- Receptor analogues is valuable tool in controlling F18+ *E. coli* infections on pig farms

- Their practical use will depend on

  -Cheap production of the sugar conjugate

  -F4+ *E. coli* receptor analogues are a next step

- A combination F18R and F4R analogues will have his place his place in strategies to control colibacilloses in newly weaned piglets

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Dr. Micheala Loos (Delevalle, Belgium)

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