

Immunoprophylactic alternatives to zinc oxide

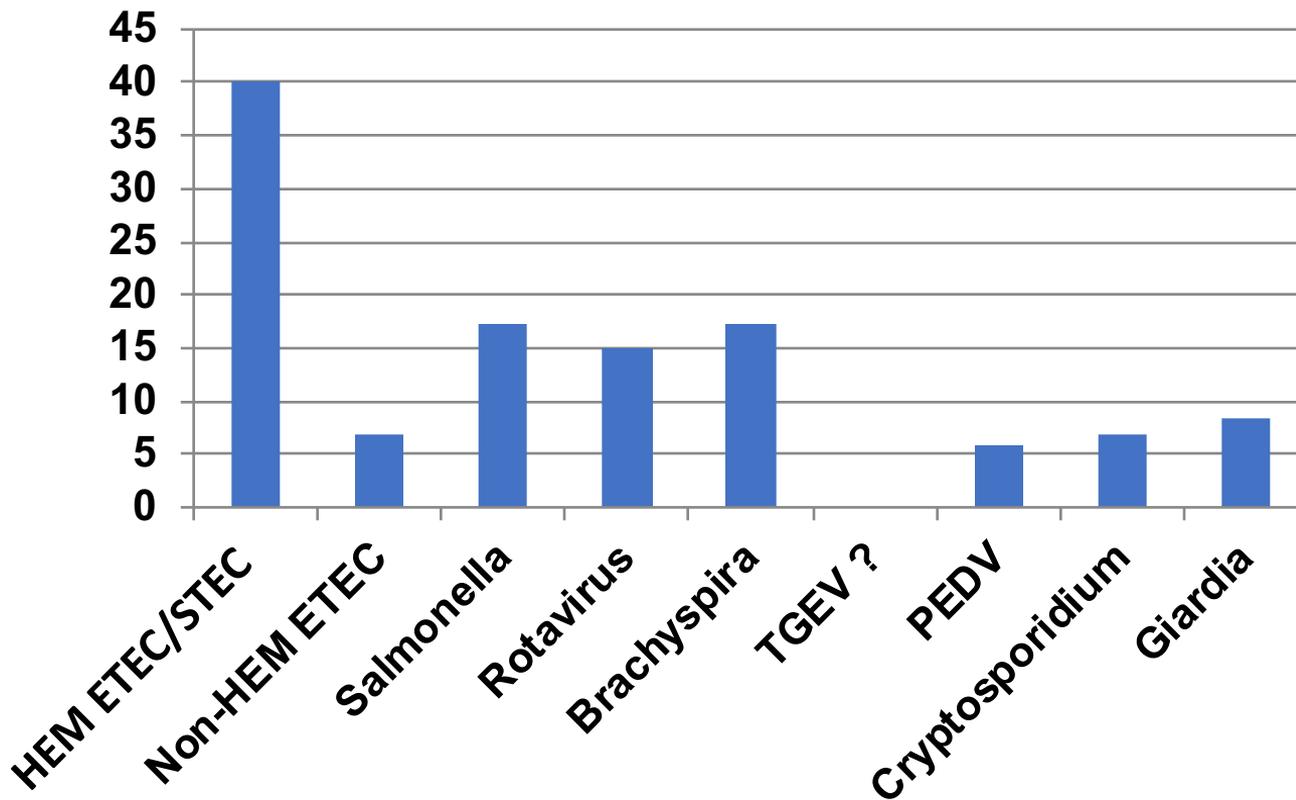


Eric Cox, Laboratory of Immunology, Fac. Vet. Med., UGent, Belgium

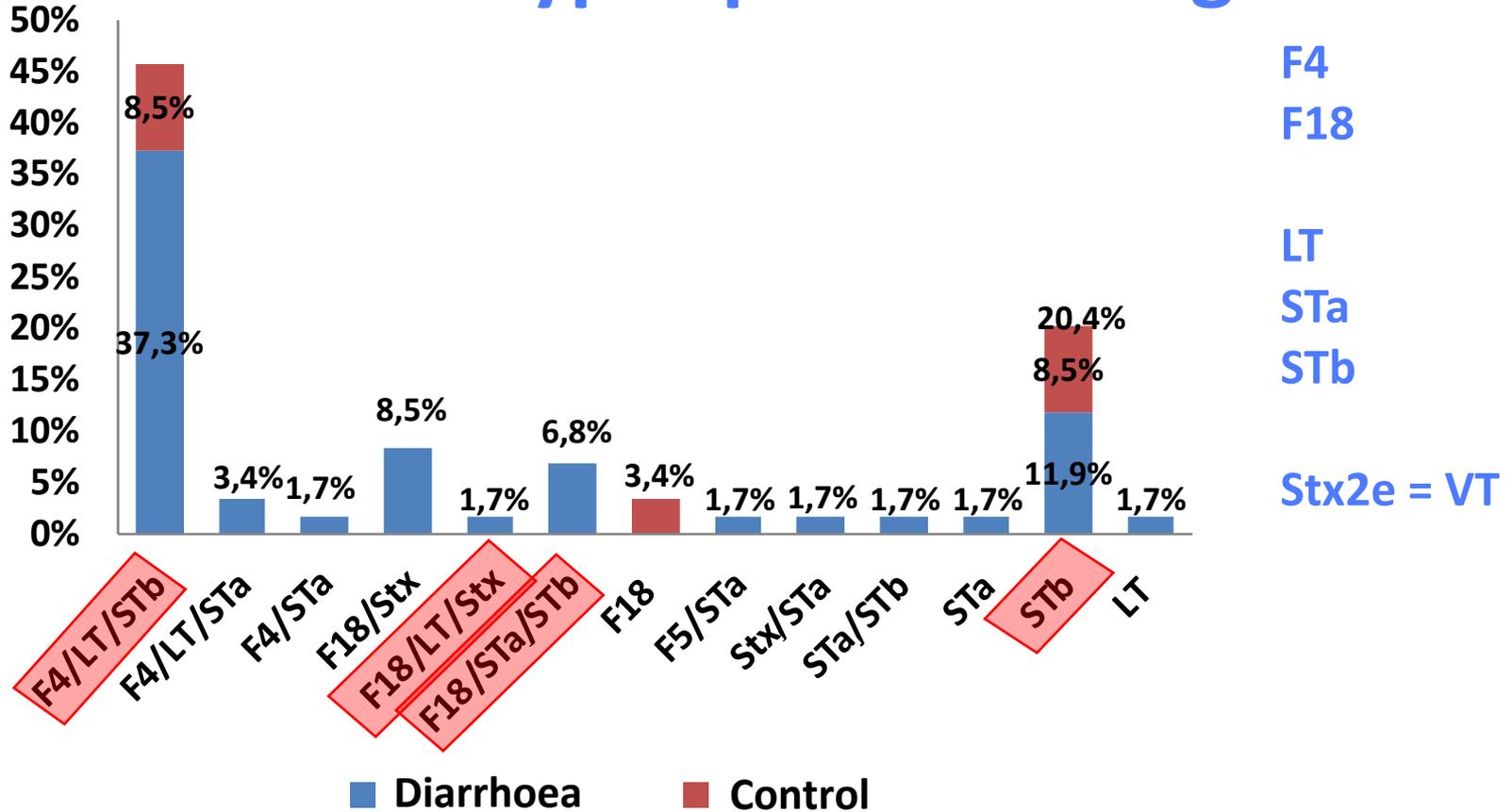
Eric.Cox@Ugent.be

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

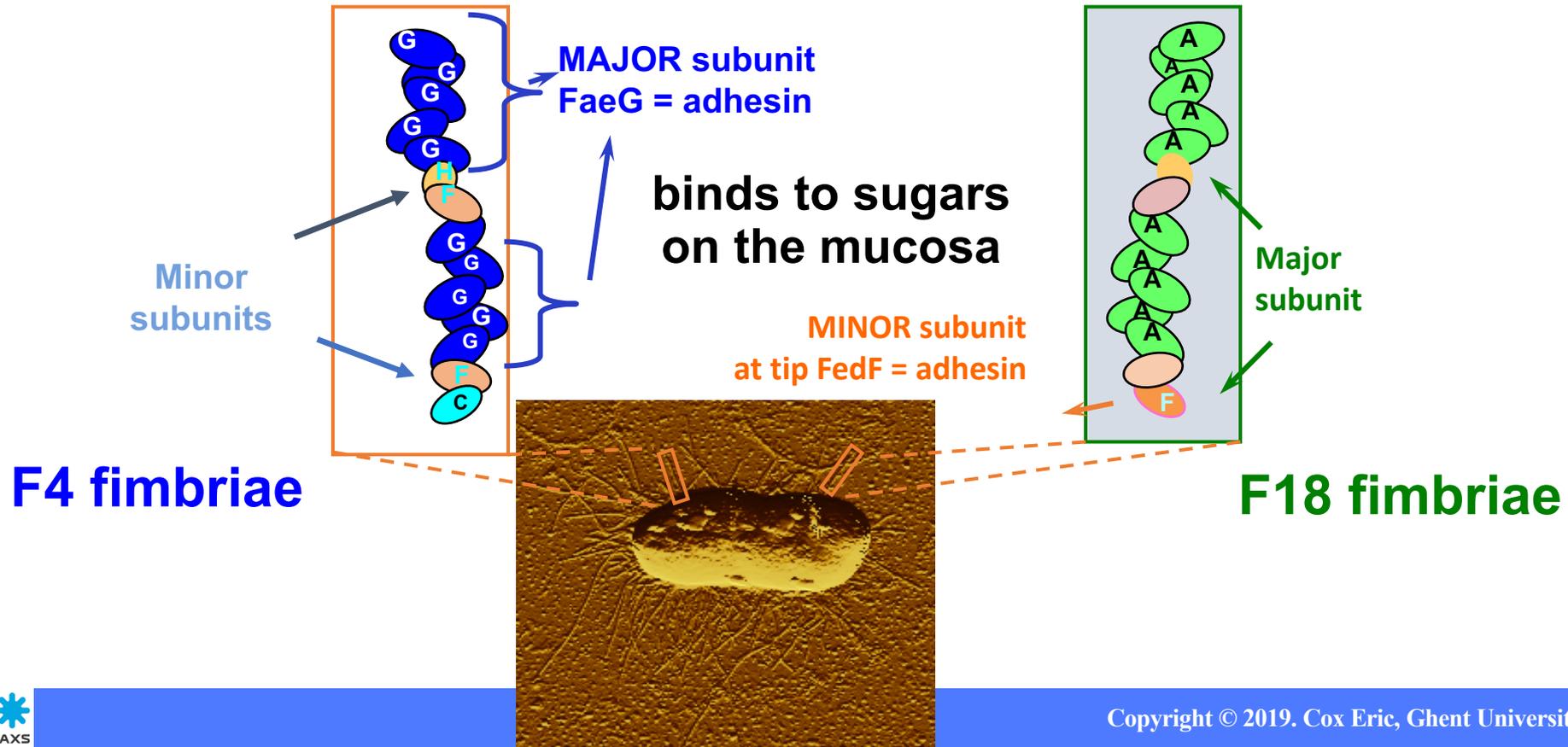
Percentage enteropathogens



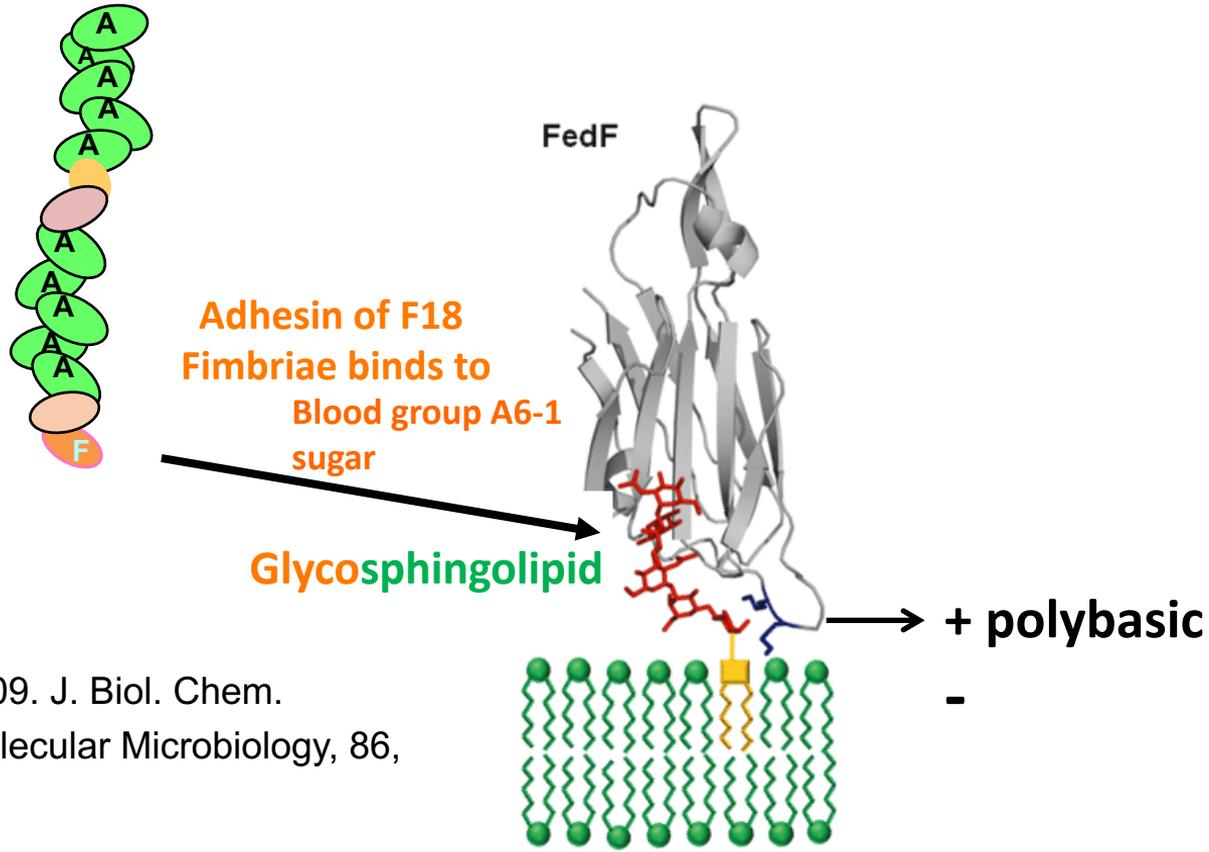
E. coli virotypes post-weaning



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



Binding of the F18 fimbrial adhesin FedF to blood group sugars shows intimate interaction with the cell

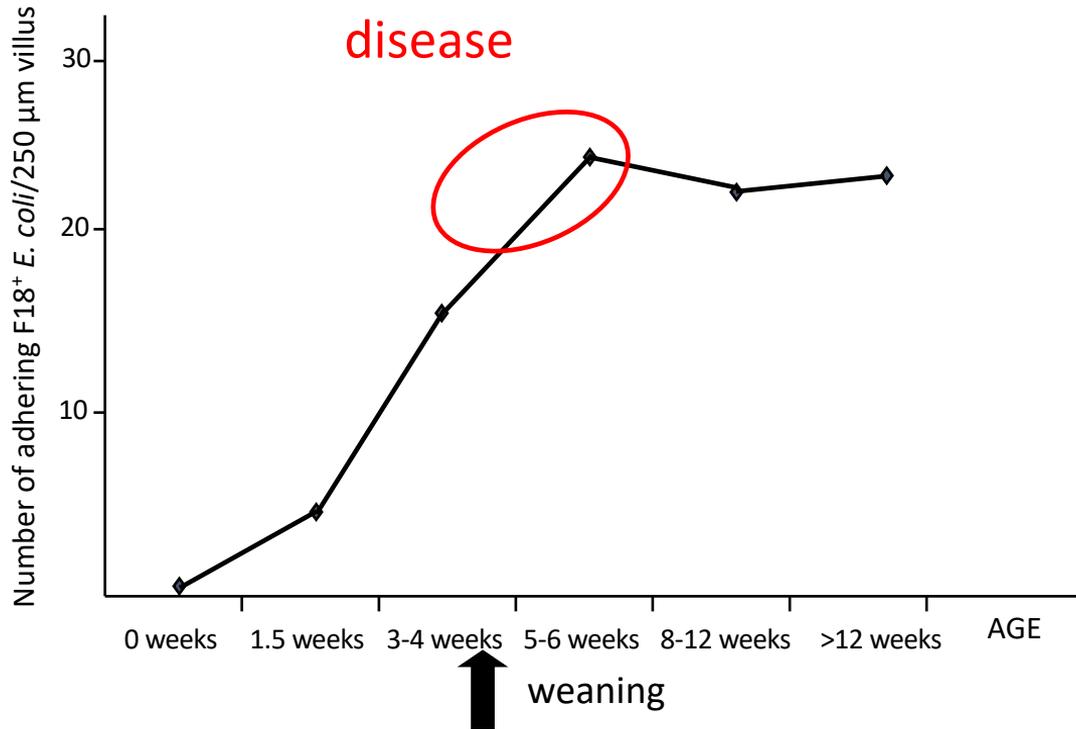


Coddens et al., 2009. J. Biol. Chem.

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

F18R expression pattern with age

=> 74 pigs aged between 0 and 23 weeks tested for F18⁺ *E. coli* adhesion

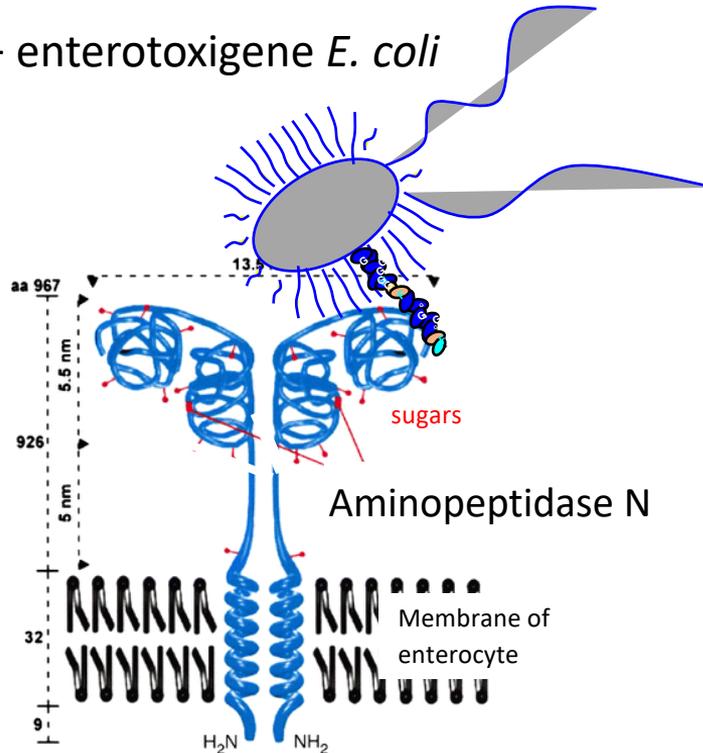


=> F18⁺ *E. coli* adhesion is age-dependent

The F4 receptors are glycoproteins

Binding occurs to the sugar and is independent of age

F4+ enterotoxigene *E. coli*



a zinc-dependent metallo-peptidase
Glycoprotein recognized by F4ac and F4ab

Enterotoxins induce diarrhoea

LT enterotoxin

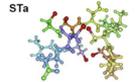
MW 84,000 Da
LTA= 28,000 Da, and 5 x LTB= 11,500 Da



- Strong toxin once cells start to secrete they secrete till they die
- Strong immunogen
- Strong adjuvant

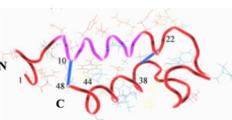
STa enterotoxin

17 AA; MW: 1,972 Da
= 42 keer lichter



STb enterotoxin

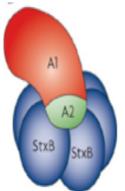
71 AA; MW: 7,604 Da
= 11 keer lichter



- Very weak immunogens
- Only induce diarrhoea when they are there
- STb also induced inflammation

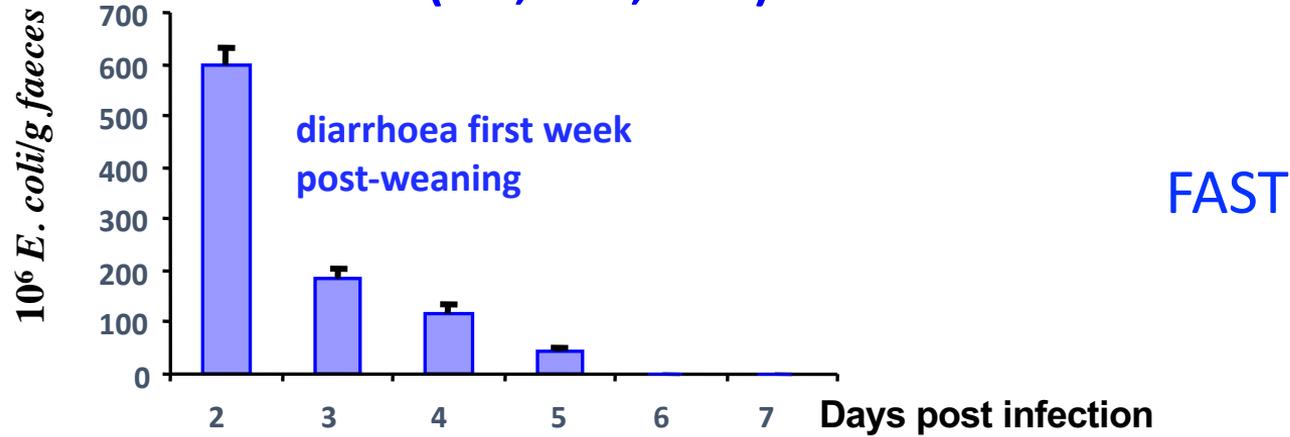
Shiga toxin induces oedema disease

Stx2e enterotoxin

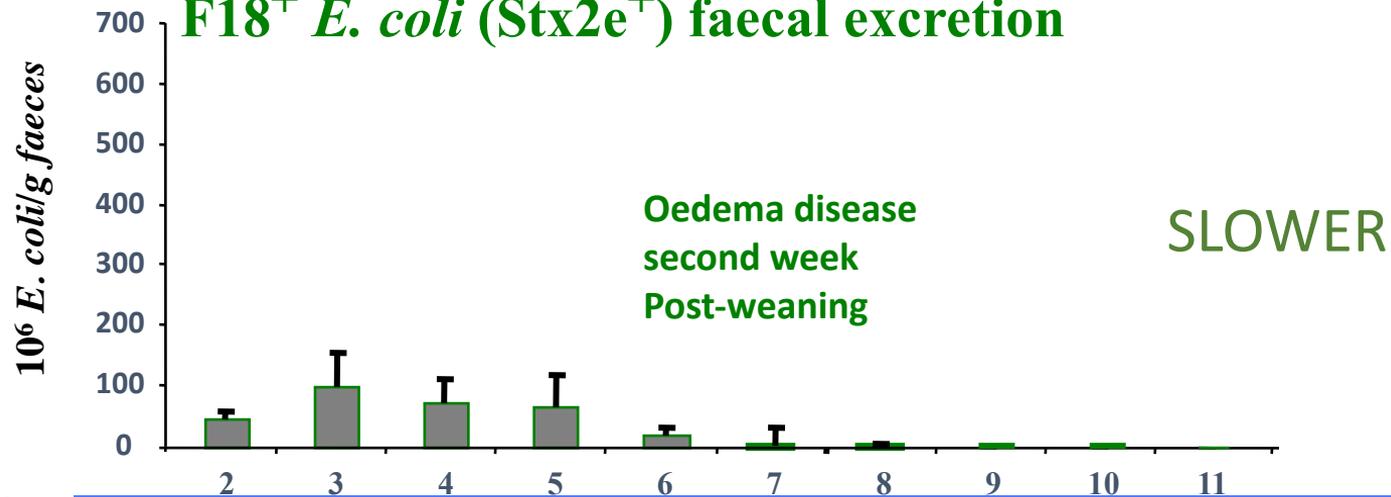


Immunogenic/
Immunosuppressive ?

F4⁺ *E. coli* (LT⁺,STa⁺,STb⁺) faecal excretion

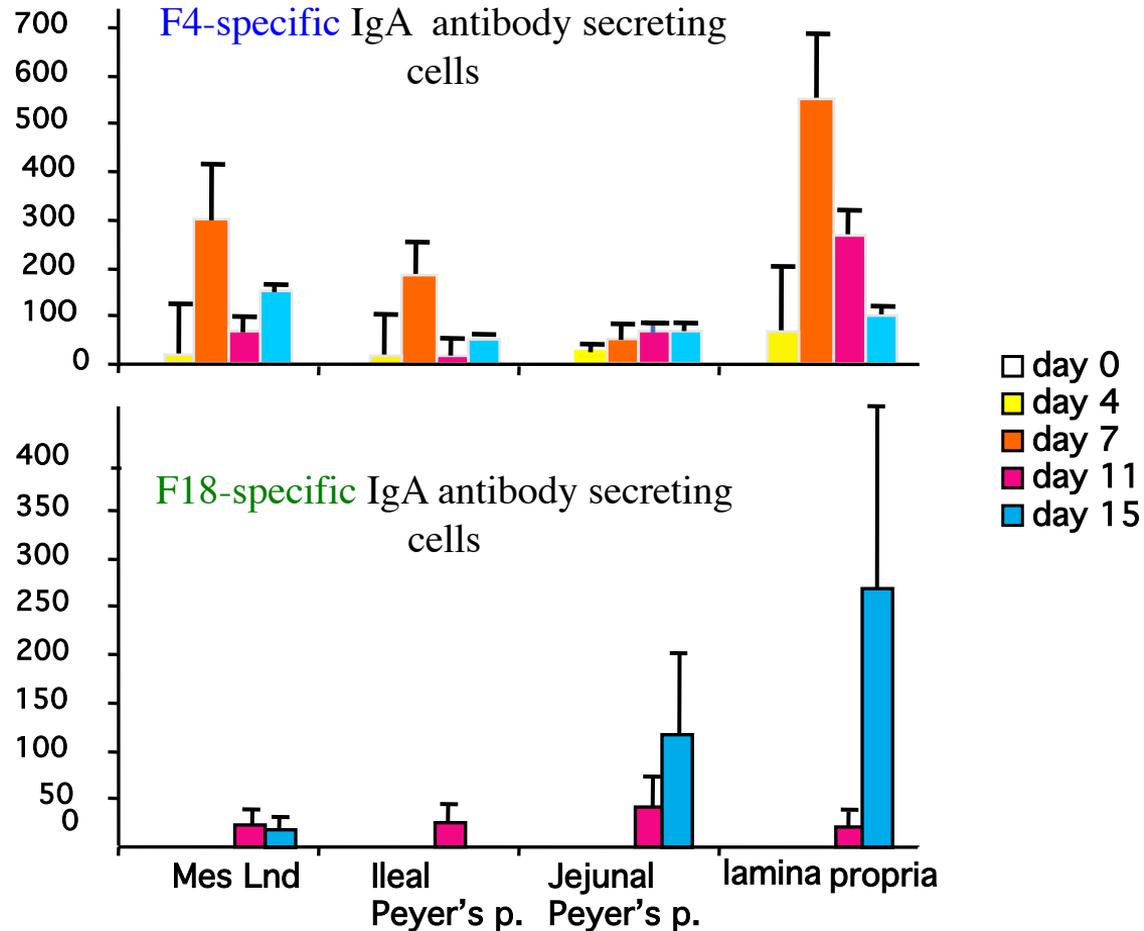


F18⁺ *E. coli* (Stx2e⁺) faecal excretion



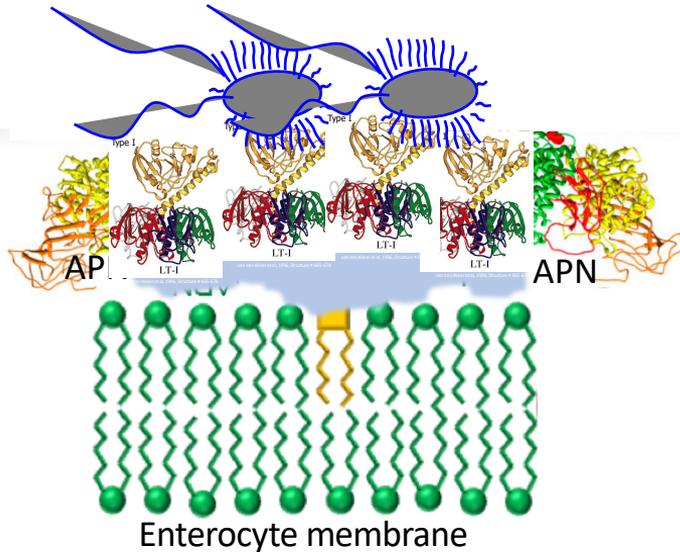
The IgA immune response clears the infection and comes earlier for F4 ETEC

Mean number of cells secreting IgA/
 10^7 cells

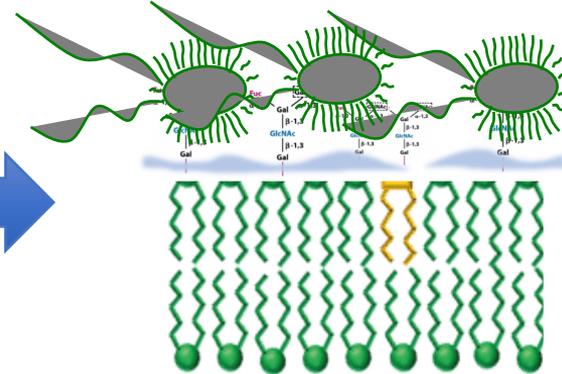


Easy colonization
 Fast colonization
 Strong immune response
 Rapidly cleared

F4+ ETEC



F18 STEC



Intimate contact
 Slow colonization
 Weaker immune response
 Stays longer

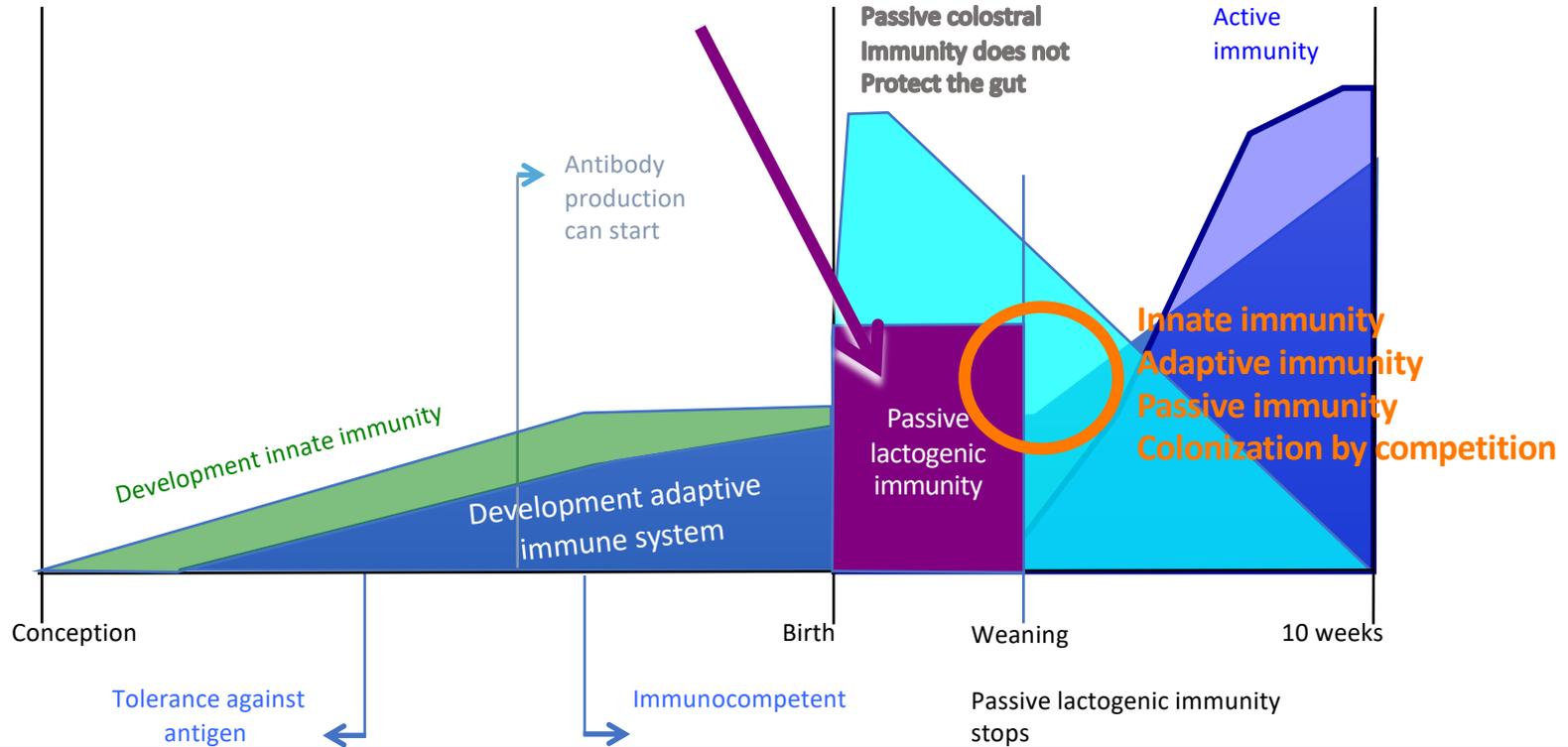
Interventions

- Induction of innate immunity: feed supplementation with β glucans
- Vaccination
- Passive immunity
- Receptor analogues
- Anti-inflammatory

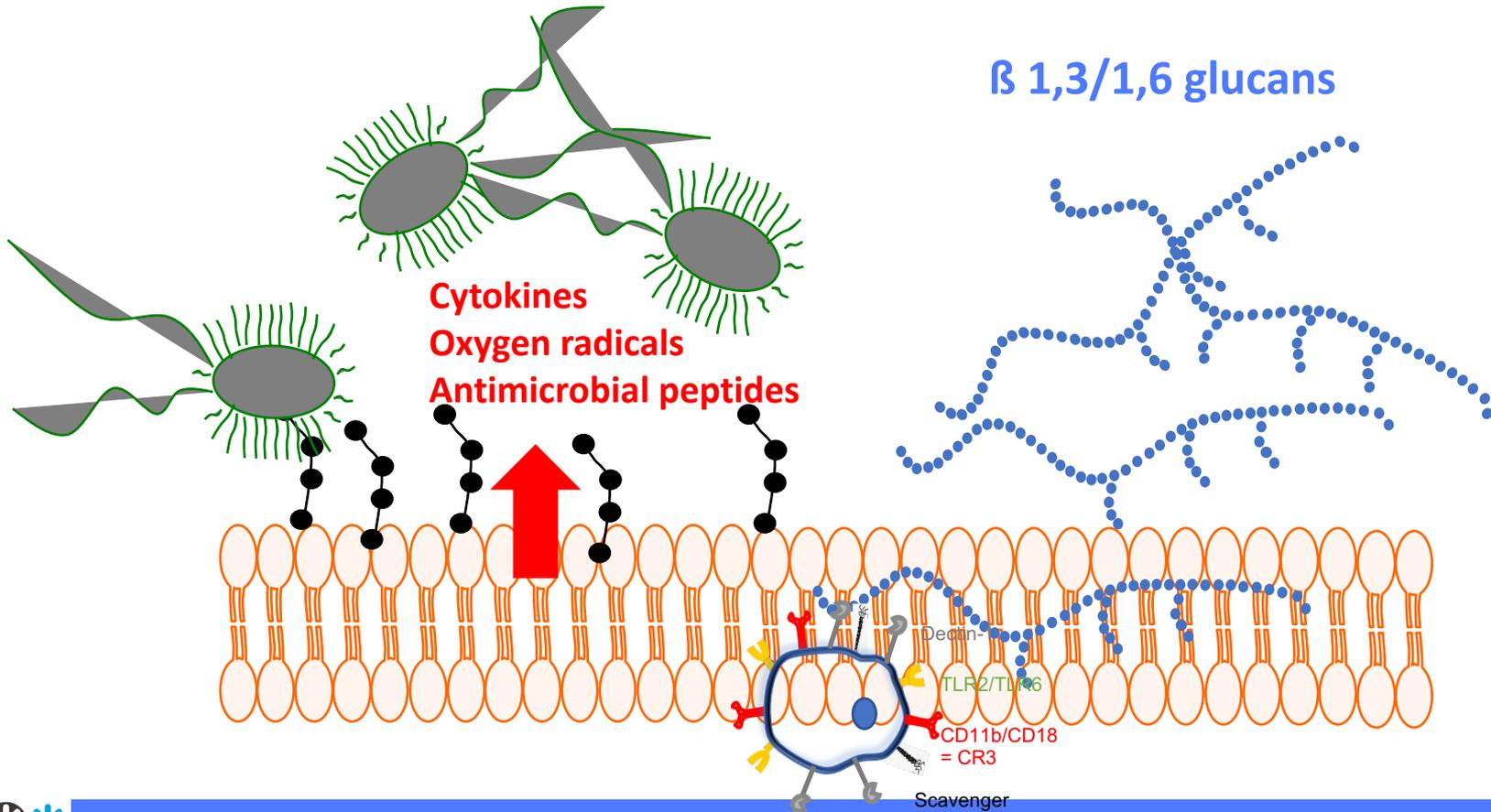
Passive Immunity and mucosal immune response

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

Cox, UGent, 2019

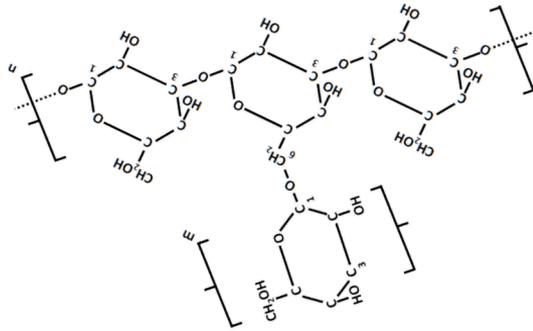


Activation of innate immunity

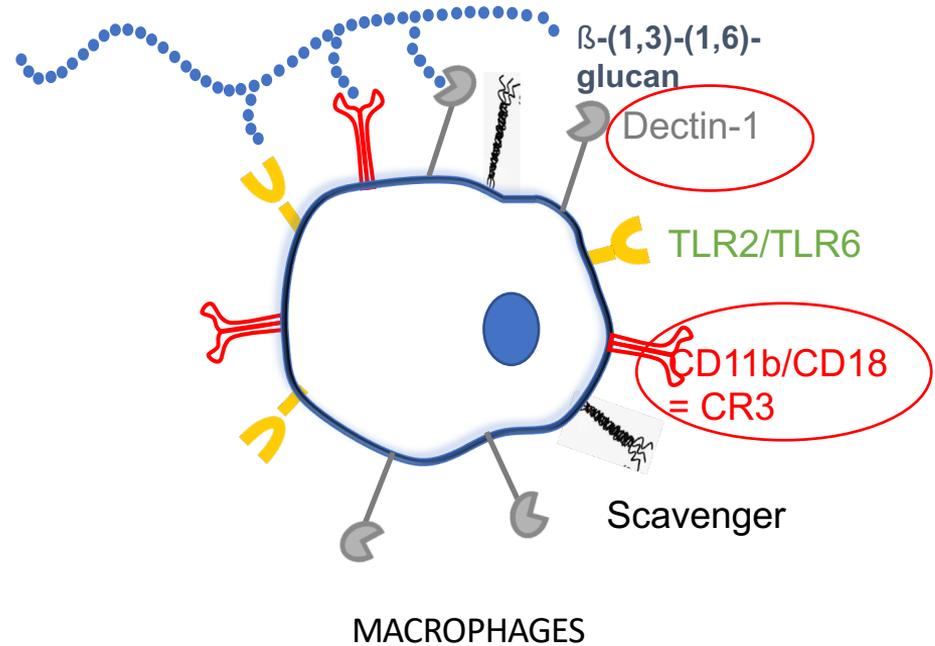


Molecules recognized by receptors of the immune system

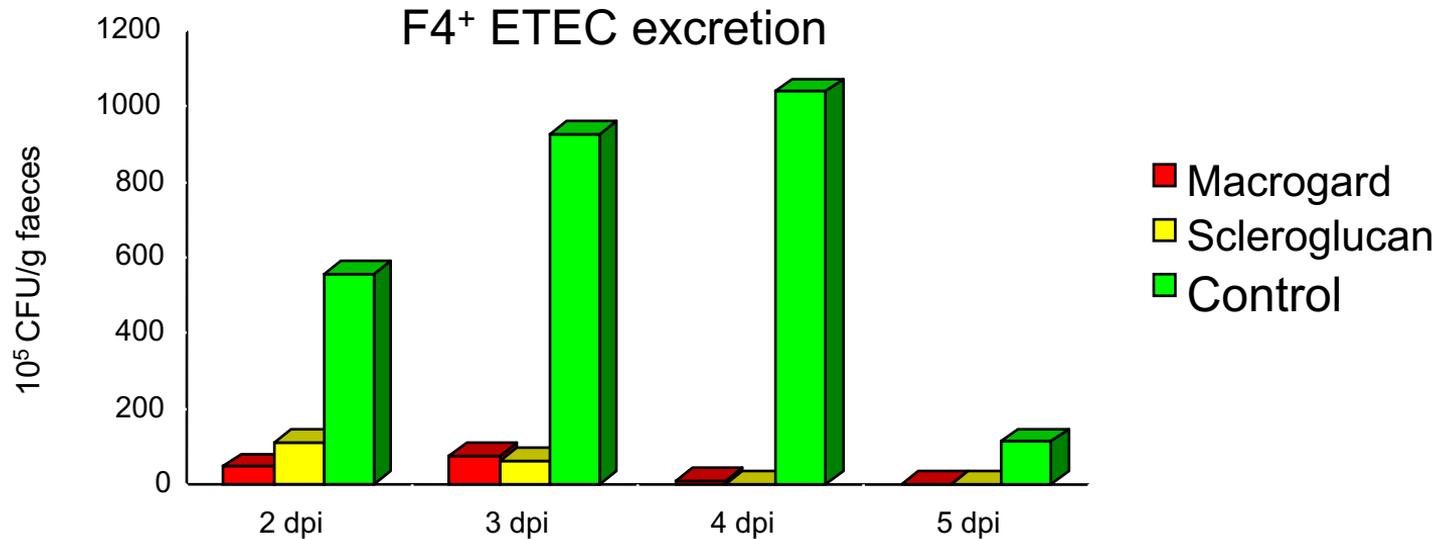
β 1,3/1,6 glucans



Pattern recognition receptors



Molecules recognized by receptors of the immune system can decrease colonization with ETEC given orally as feed supplement

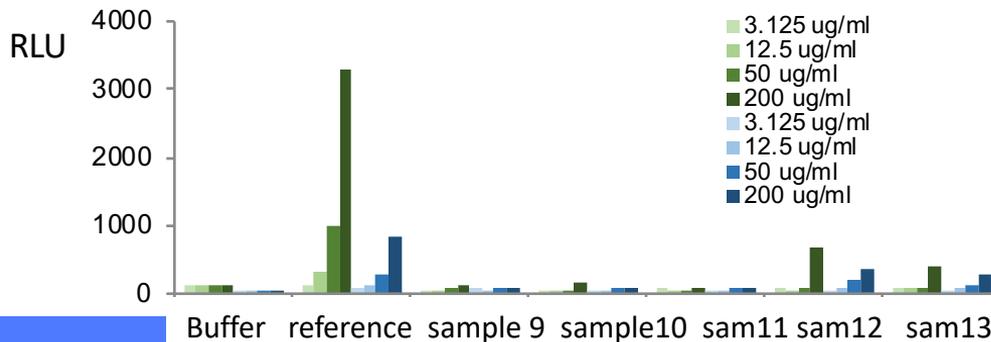
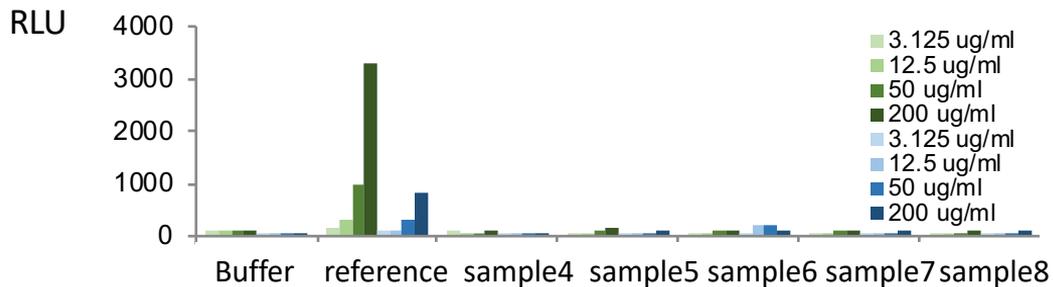
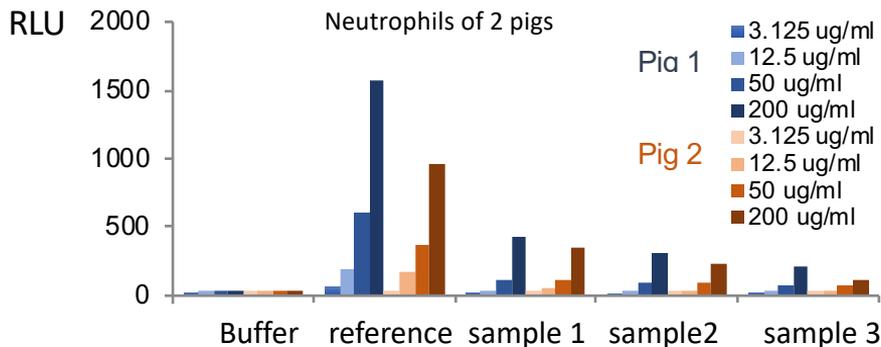


Sufficiently long > 10d

High enough but not too high: 500 ppm

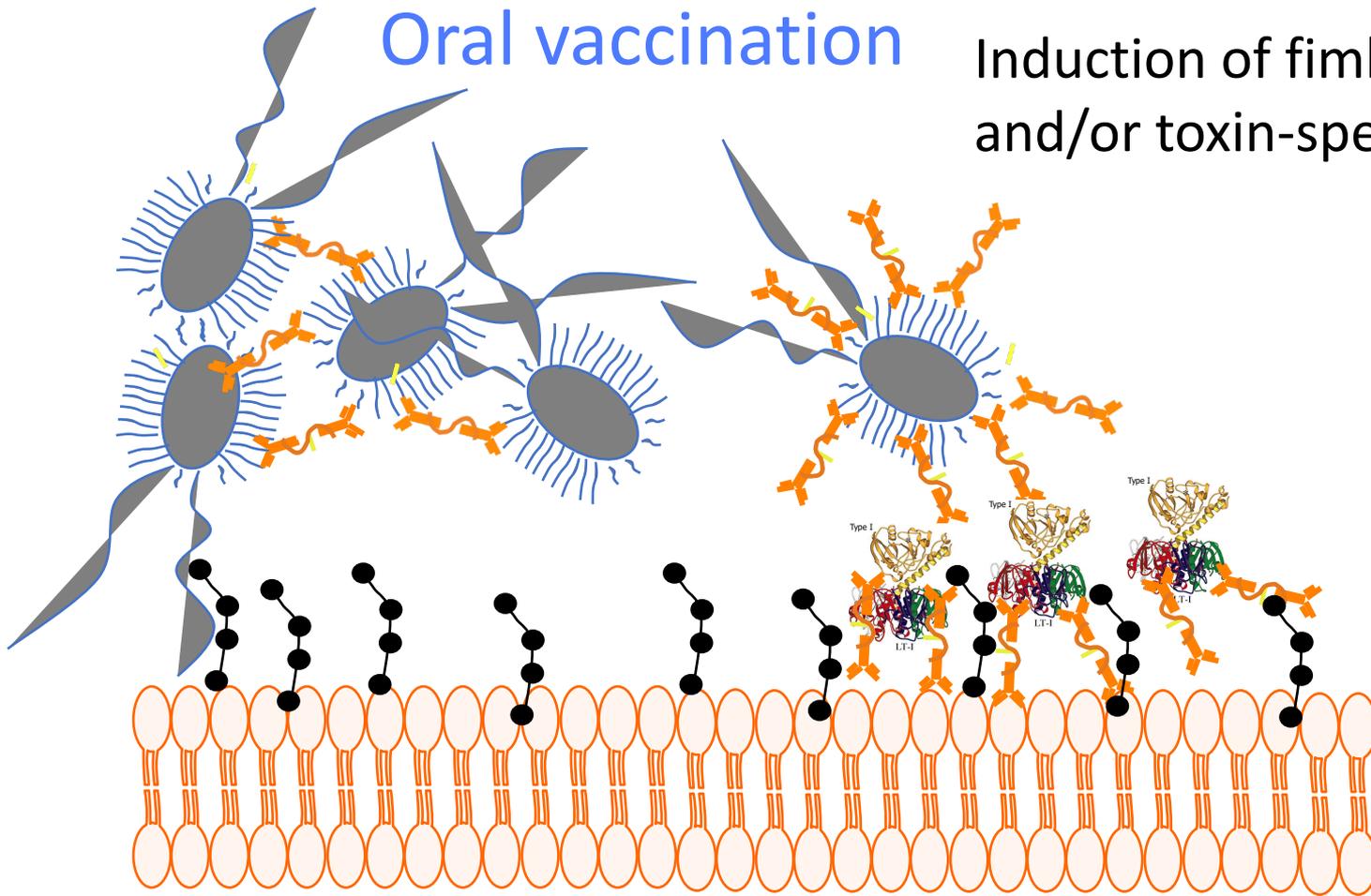
Sufficiently pure

Neutrophils' ROS-production as measure of quality of Glucan preparations

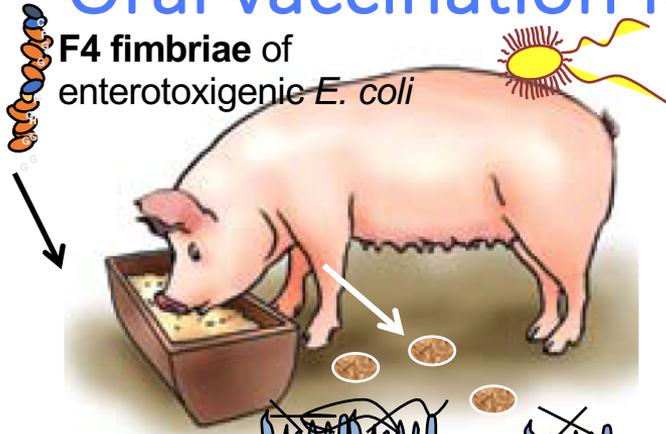


Oral vaccination

Induction of fimbriae- specific
and/or toxin-specific SIgA

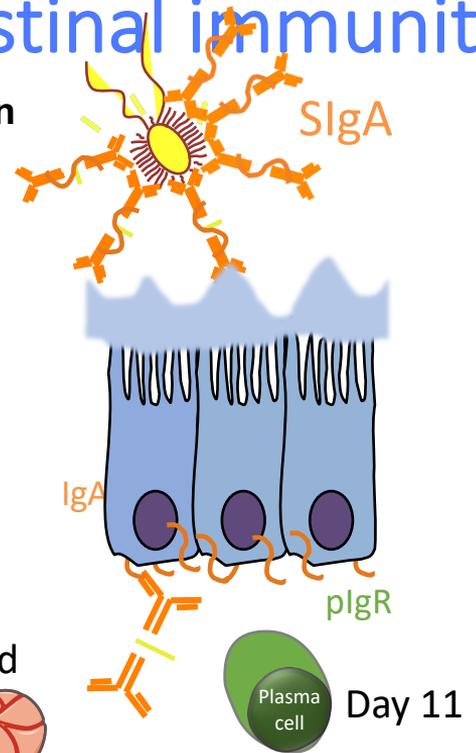


Oral vaccination for induction of intestinal immunity



Van den Broeck et al., 1999. Vaccine

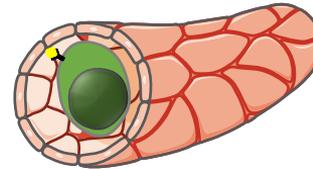
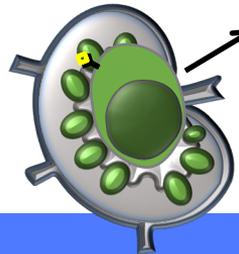
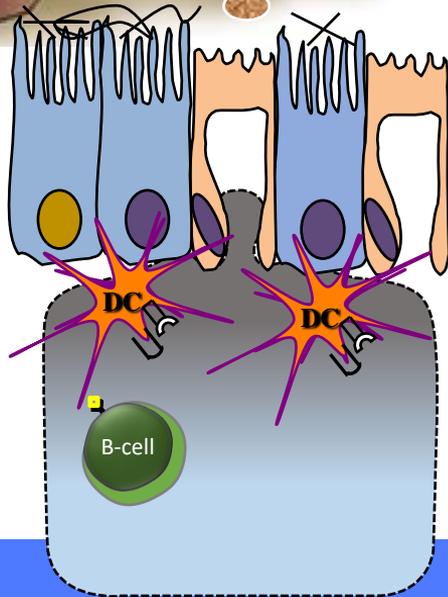
Protection



Day 9 Blood

Day 7
Mes Lymph n.

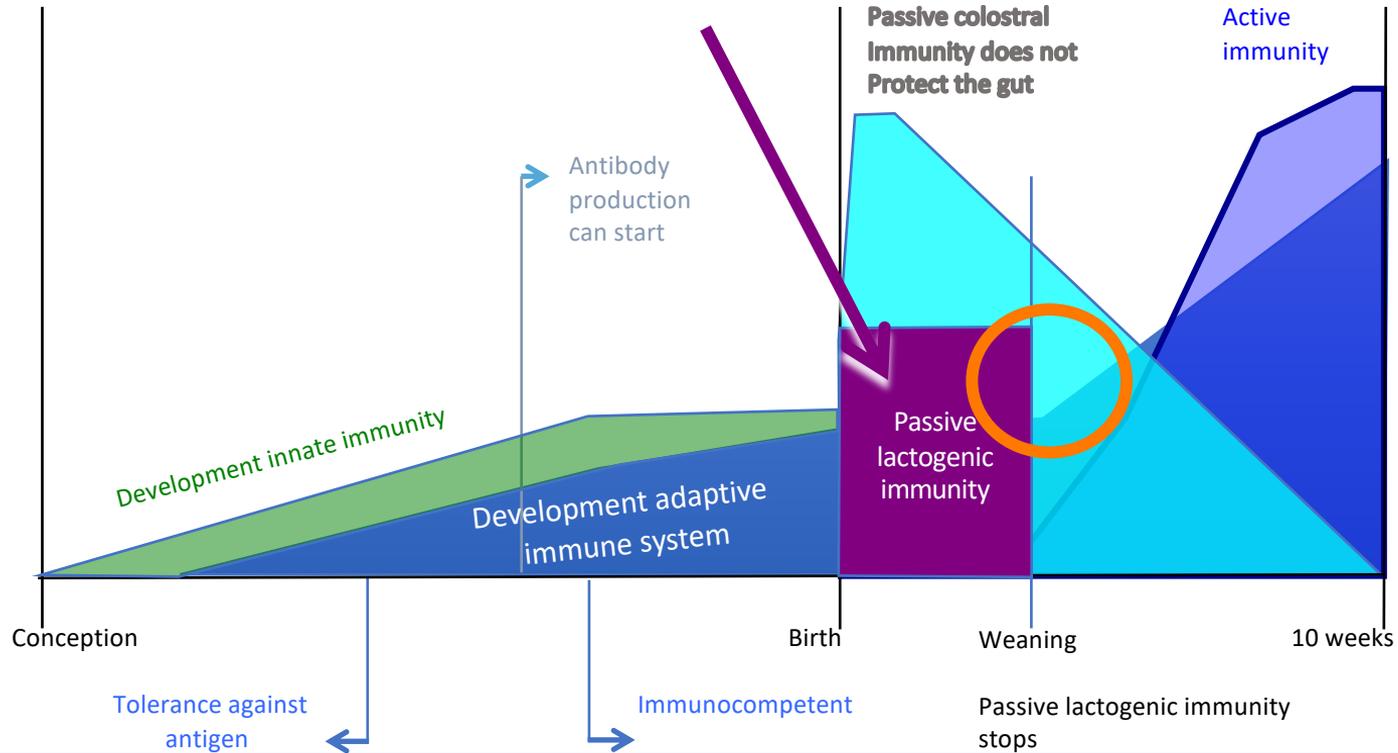
Day 4
Peyer's
patches



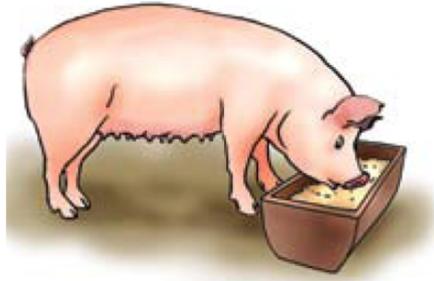
Passive Immunity and mucosal immune response

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

Cox, UGent, 2019



Different strategies are followed



F4 fimbriae

- Carrier (Tiels et al, 2008. Vaccine)



Anti-APN antibodies targeting soluble antigen to APN and the GALT

- Porcinized Monoclonal Ab (Van de Weken et al., 2019. Mabs)

Coliprotect F4/F18 live oral vaccine : *E. coli* O8:K87 / O141:K94 vaccine

Leaflet:

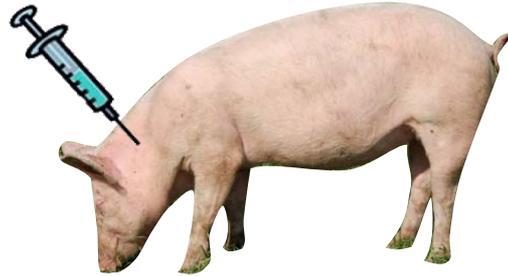
- Oral once directly in mouth or in drinking water
- Effective in seronegative piglets, 17 days old, without maternal immunity, nor suckling, 7 days post vaccination
- Protection until at least 21 days post immunisation

Experiment	Group	Number of animals	Treatment (lot number)	Vaccine dose (CFU)	Vaccination-challenge interval (days)
7-dpv challenge	Vaccine	10	Coliprotect [®] F4 (121811)	1.3×10^8	7
	Control	10	Water	Not applicable	7
21-dpv challenge	Vaccine	10	Coliprotect [®] F4 (121811)	1.3×10^8	21
	Control	10	Water	Not applicable	21
3-dpv challenge	Vaccine	20	Coliprotect [®] F4 (122964)	5.9×10^7	3
	Control	20	Water	Not applicable	3

What in the presence of milk antibodies (lactogenic immunity) that neutralize the pathogen?

Vaccination against the toxine

Stx2e
toxoid

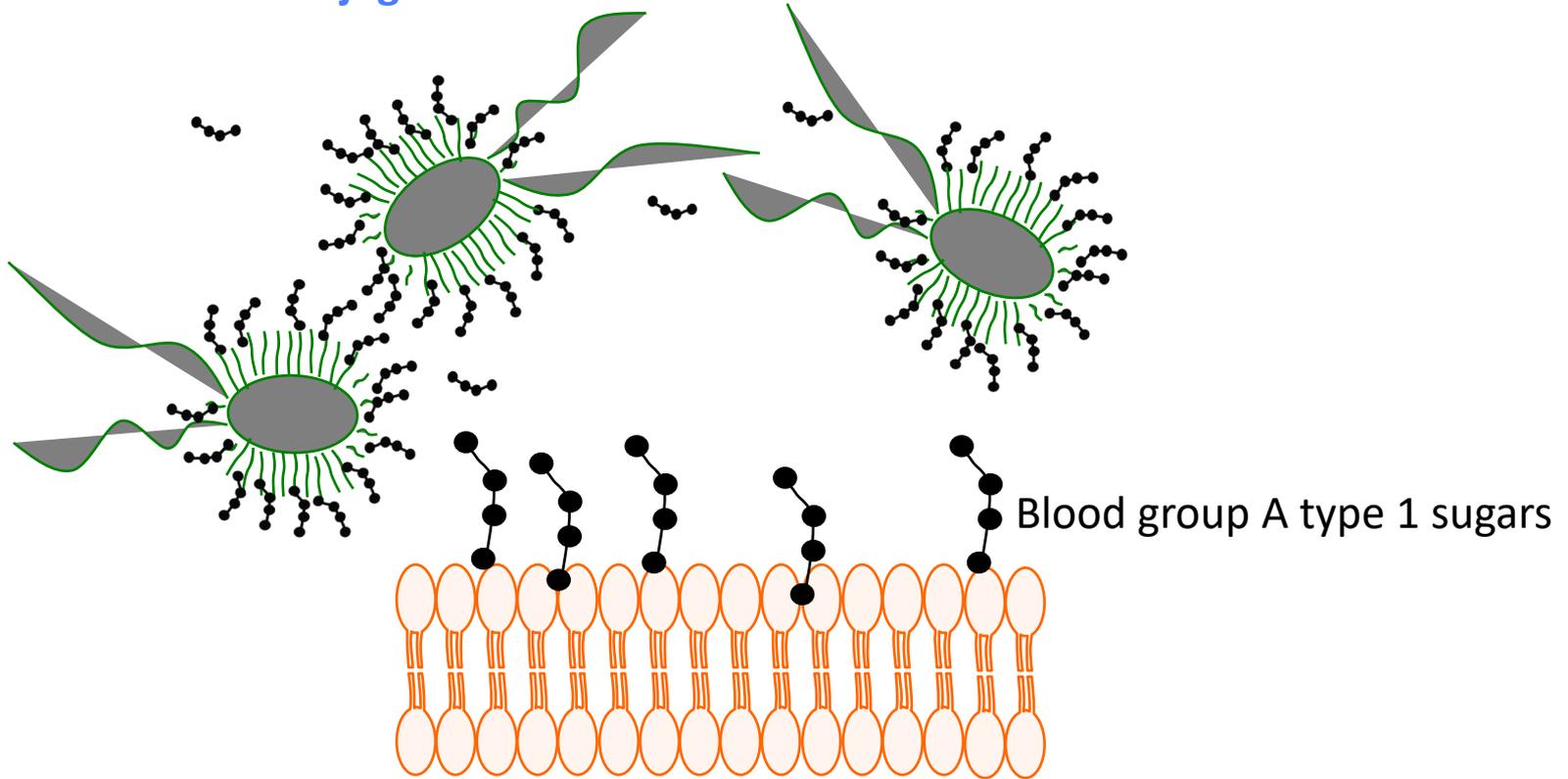


Anti-Stx2e
neutralizing
antibodies

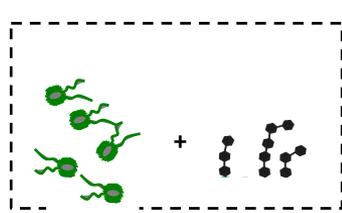
- Stx2e toxoid immunogen is safe
- Good Stx2e antibody responses after immunization on piglet
- Immunization with Stx2e toxoid protect piglets from action of Stx2e toxin (causing clinical signs and lesions of ED)
- Two registered vaccines:
 - VEPURED (HIPRA) : starting 2 days of age IM, AIOH, DEAE-dextran, immunity after 3w during 4 m
 - ECOPORC SHIGA (IDT) : starting 4 days of age IM, AIOH, immunity after 3 weeks during 3 ½ maand

Receptor analogues

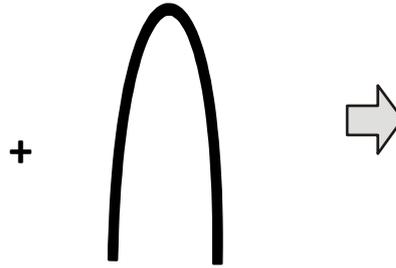
A6-conjugate decreases colonization with F18 *E. coli*



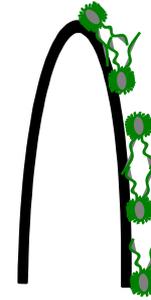
In vitro villus adhesion to study interaction of *E. coli* with the villi and inhibition by molecules



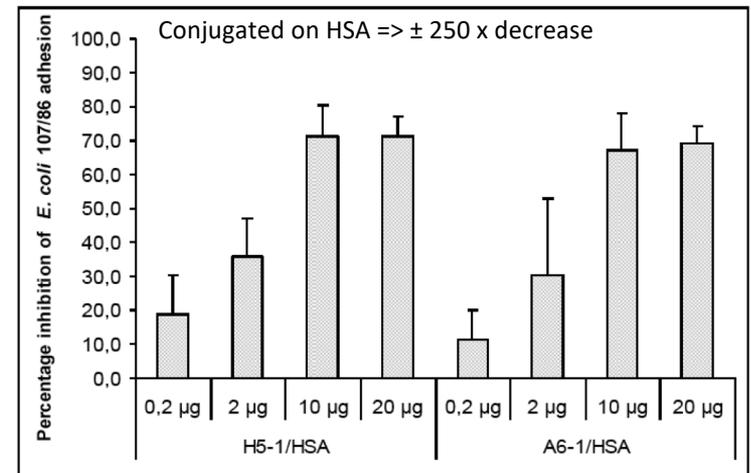
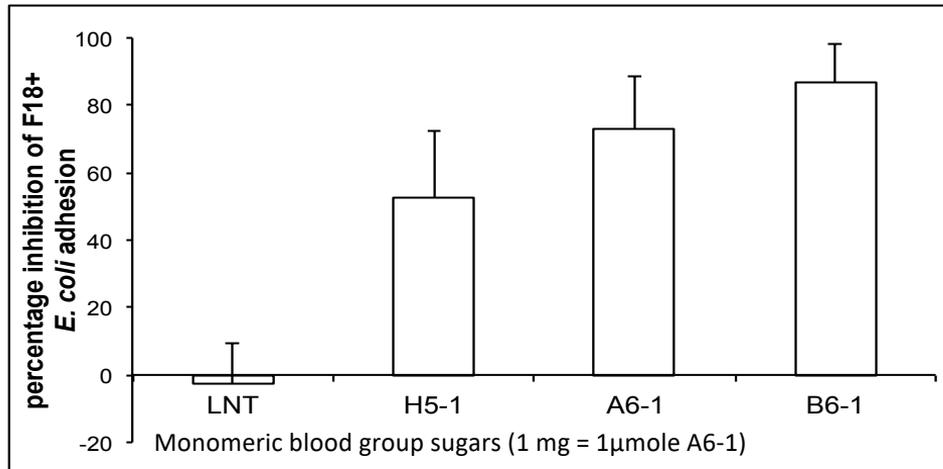
Pre-incubation of bacteria with H5-1, A6-1, B6-1 sugars



Are added to small intestinal villi of F18R+ piglets

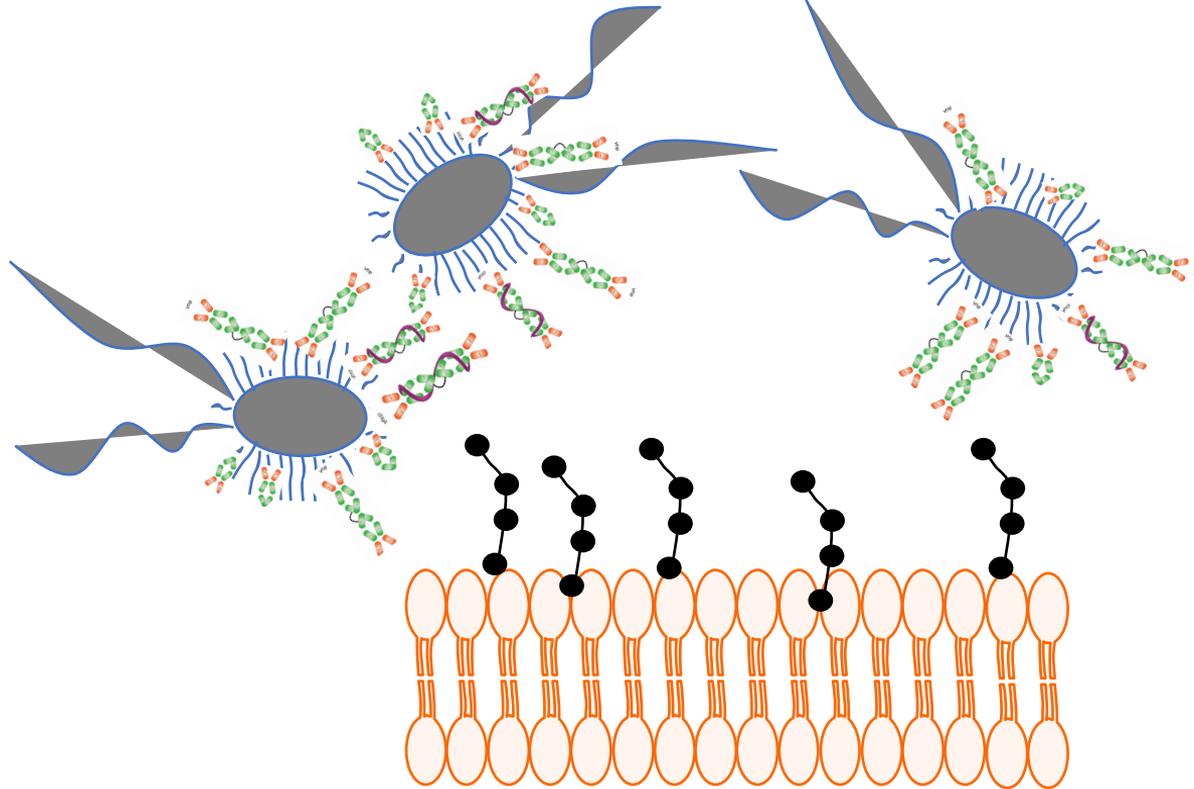


Percent inhibition of adhesion is calculated ?

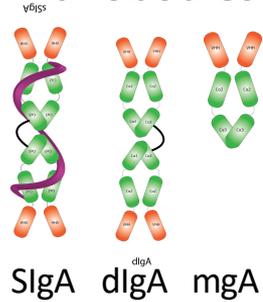


Antibodies via feed to prolong passive immunity

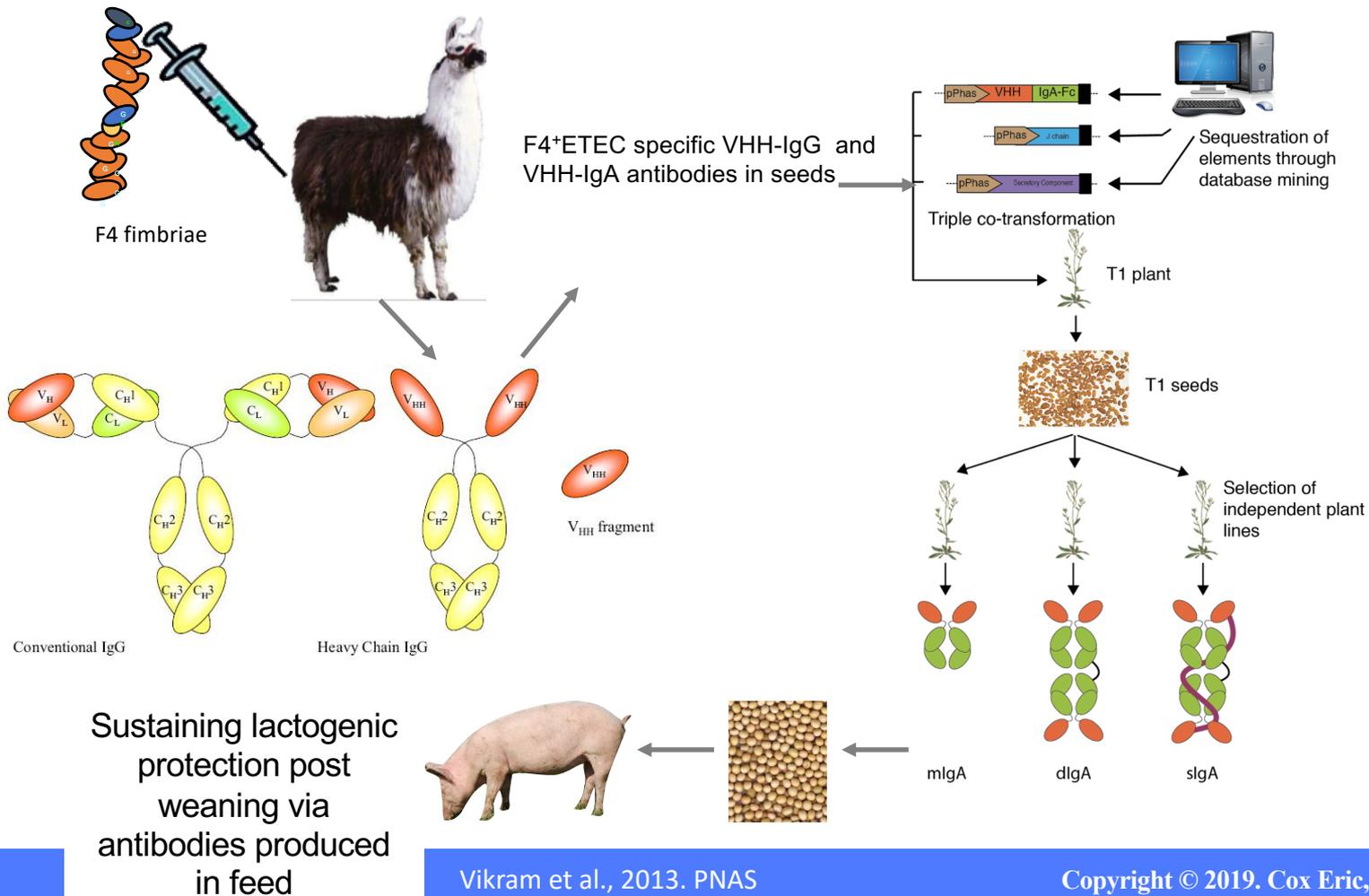
Porcinized Nanobodies

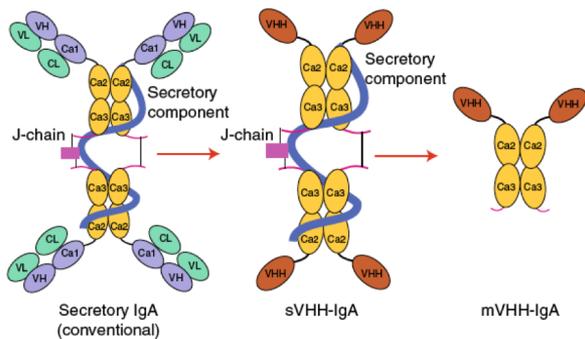


Nanobodies

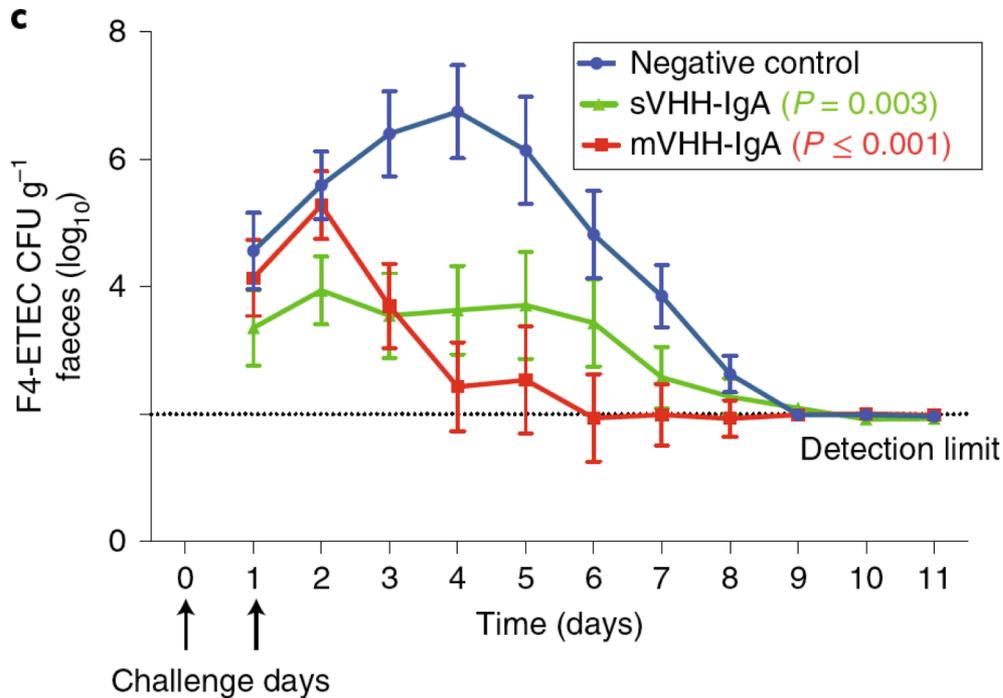


NANOBODY THERAPY = passive immunity in feed





Yeast-secreted, dried and food-admixed monomeric IgA prevents gastrointestinal infection in a piglet model



Conclusion

- 1. Different strategies exist or are in development**
- 2. There is currently no optimal alternative compared to antibiotics**
- 3. Management combined with several other strategies are needed to control colibacillosis**