

# The age-dependent expression of the F18<sup>+</sup> *E. coli* receptor on porcine gut epithelial cells is positively correlated with the presence of histo-blood group antigens

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## Abstract

F18<sup>+</sup> *Escherichia coli* have the ability to colonize the gut and cause oedema disease or post-weaning diarrhoea by adhering to specific F18 receptors (F18R) on the porcine epithelium. Although it is well established that a DNA polymorphism on base pair 307 of the *FUT1* gene, encoding an  $\alpha(1,2)$ fucosyltransferase, accounts for the F18R phenotype, the F18R nature is not elucidated yet. The aim of the present study was to investigate the correlation between the presence of H-2 histo-blood group antigens (HBGAs) or its derivative A-2 HBGAs on the porcine gut epithelium and F18<sup>+</sup> *E. coli* adherence. A significant positive correlation was found between expression of both the H-2 ( $r = 0.586$ ,  $P < 0.01$ ) and A-2 ( $r = 0.775$ ,  $P < 0.01$ ) HBGAs and F18<sup>+</sup> *E. coli* adherence after examination of 74 pigs aged from 0 to 23 weeks. The majority of the genetically resistant pigs (*FUT1M307<sup>A/A</sup>*) showed no HBGA expression (91.7%) and no F18<sup>+</sup> *E. coli* adherence (83.3%). In addition, it was found that F18R expression levels rise with increasing age during the first 3 weeks after birth and that F18R expression is maintained in older pigs (3–23 weeks old). Taken together, these data suggest that, apart from H-2 HBGAs, A-2 HBGAs might be involved in F18<sup>+</sup> *E. coli* adherence.

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## 1. Introduction

Newly weaned pigs are very susceptible to F18 positive *Escherichia coli* (F18<sup>+</sup> *E. coli*) infections leading to post-weaning diarrhoea or oedema disease. In this infection process, the F18 receptor (F18R) plays

a crucial role by mediating the binding of F18 fimbriated bacteria to the intestinal epithelium. This leads to colonization of the gut and subsequent secretion of entero- or verotoxins. The population of pigs consists of F18R negative (F18R<sup>−</sup>) and F18R positive (F18R<sup>+</sup>) animals and only the latter are subject to infection with F18<sup>+</sup> *E. coli* (Frydendahl et al., 2003). The F18R status of pigs is genetically determined (Bertschinger et al., 1993) and susceptibility to F18<sup>+</sup> *E.*

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*coli* infections appeared to be dependent on the activity of the *FUT1* gene, encoding the alpha(1,2)-fucosyltransferase (FUT1) (Meijerink et al., 1997, 2000). Sequencing of the *FUT1* gene of pigs resistant to F18<sup>+</sup> *E. coli* infection revealed a transition (G→A) of both alleles on bp 307. This results in an amino acid substitution at position 103 (Ala→Thr) in the resistant *FUT1M307<sup>A/A</sup>* pigs in contrast with the susceptible *FUT1M307<sup>G/A</sup>* or *FUT1M307<sup>G/G</sup>* pigs.

Alpha(1,2)-fucosyltransferases (FUTs) are key enzymes involved in the formation of blood group antigens of the porcine AO blood group system, which corresponds to the human ABO blood group system (Sako et al., 1990). FUT transfers a fucose to H precursors (Galβ1,3/4GlcNAcβ1-R) leading to the synthesis of H blood group antigens (Galβ1,3/4(Fucα1,2)GlcNAcβ1-R). Expression of A blood group antigens ((GalNAcα1,3)Galβ1,3/4(Fucα1,2)GlcNAcβ1-R) is due to the action of an *N*-acetylgalactosaminyltransferase that mediates the addition of GalNAc to the H antigen. As in humans, the phenotypic appearance of the A blood group antigens in pigs is dependent on the expression of its precursor, the H antigen. Expression of blood group antigens is not confined to erythrocytes (Landsteiner, 1900), as they are also present on the cell surfaces of endothelial and a variety of epithelial cells even as in secretions (Ravn and Dabelsteen, 2000; Marionneau

et al., 2001). Therefore, they are termed 'histo-blood group antigens' (HBGAs) (Clausen and Hakomori, 1989).

It has been shown earlier that H type 2 (H-2) (Galβ1,4(Fucα1,2)GlcNAcβ1-R) or A type 2 (A-2) ((GalNAcα1,3)Galβ1,4(Fucα1,2)GlcNAcβ1-R) blood group antigens can be expressed on porcine intestinal epithelium (King and Kelly, 1991). In addition, it was found that H-2 HBGAs might be involved in F18<sup>+</sup> *E. coli* adhesion (Snoeck et al., 2004), however, the role of A-2 antigens in F18<sup>+</sup> *E. coli* adhesion has not been investigated before.

## 2. Materials and methods

### 2.1. Pigs

In this study, 74 pigs of 33 different litters (Belgian Landrace × Piétrain or Dutch landrace) of 4 different farms were examined and subdivided in 13 groups of different ages (Table 1). Each group contains at least three pigs out of at least two different litters. The newborn piglets (category 0 weeks old) were deprived of colostrum and euthanized maximum 4 h after birth, whereas the pigs of 1.5 and 3 weeks old received sow's milk ad libitum on the farm. After purchase and transport to isolation units, they received water and

Table 1  
Summary of data on the age-dependent expression of the F18R and HBGAs

Age weeks	Number of pigs	<i>FUT1M307</i>			F18 <sup>+</sup> <i>E. coli</i> adherence per 250 µm villus			HBGA expression							
		G/G	G/A	A/A	<5	5–30	>30	H-2 positive				A-2 positive			
								–	+	++	+++	–	+	++	+++
0	4	1	2	1	4			4				2	2		
1.5	4	3	1		3	1			2	2		4			
3	12	4	8		5	5	2	7		4	1	8		2	2
4	5	2	2	1	3		2	2		1	2	5			
5–6	8	8				6	2	5		1	2	4		1	3
8	5	3	2		2	2	1	2			3	4			1
9	6	3	2	1		6		3			3	6			
10–11	5	1	2	2	2	1	2	3	1		1	3			2
12	5	3	2			3	2	2			3	3			2
13	8	6	2		3	5		5		1	2	7			1
14	5	3	1	1		3	2	5					1		4
17–18	4	2	2				4	4							4
22–23	3	2	1		1		2	1			2	3			

Abbreviations: A = adenine, G = guanine, *FUT1M307*: basepair 307 of the *FUT1* gene.

Table 2

Overview of data on F18<sup>+</sup> *E. coli* adhesion and blood group antigen expression of 12 pigs with the *FUT1M307<sup>A/A</sup>* genotype

Pig	Age (weeks)	F18 <sup>+</sup> <i>E. coli</i> adherence per 250 µm villus	HBGA expression
1	0	0	–
2	4	0	–
3	9	6.2	–
4	10	1.5	–
5	11	1.2	–
6*	12	0.8	–
7	14	26.5	Very weak A-2 positive
8*	14	0	–
9*	16	0	–
10*	16	1.8	–
11*	19	0	–
12*	21	0	–

Pigs that were included later are marked with an asterisk.

Baby-star pellets (NV Versele-Laga, Deinze, Belgium) ad libitum until they were euthanized 1 or 2 days later. From the age of 4 weeks, the pigs were routinely weaned and fed ad libitum (Pigo-star, NV Versele-Laga, Deinze, Belgium). In addition, six extra pigs (Belgian Landrace × Piétrain), genetically resistant to F18<sup>+</sup> *E. coli* infections, were selected out of two different litters and analyzed for F18R expression (Table 2). Euthanasia was performed by intravenous injection of an overdose of pentobarbital (24 mg/kg; Nembutal; Sanofi Santé Animale, Brussels, Belgium). The experimental and animal management procedures were undertaken in accordance with the requirements of the animal care and ethics committee of the Faculty of Veterinary Medicine (EC2004/32).

## 2.2. Bacterial inoculum

The verotoxigenic F18 positive reference strain 107/86 (serotype O139:K12:H1, F18ab<sup>+</sup>, SLT-IIv<sup>+</sup>) was cultured on BHI agar plates (Oxoid, Basingstoke, Hampshire, England) at 37 °C for a period of 18 h. The bacteria were harvested by centrifugation and suspended in PBS with 1% D-mannose whereafter the concentration of the bacteria was determined by measuring the optical density of the bacterial suspension at 660 nm (OD<sub>660</sub>). An OD of 1 equals 10<sup>9</sup> bacteria per ml, as determined by counting colony forming units.

## 2.3. Determination of the F18R genotype

Pigs were genotyped using a PCR-RFLP test based on the G→A polymorphism at nucleotide 307 of the *FUT1* gene according to Meijerink et al. (1997). Briefly, a fragment of the *FUT1* gene was amplified using specific primers (M307F: 5'-CTTCCTGA-ACGTCTATCAAGACC-3', M307R: 5'-CTTCAGC-CAGGGCTCCTTTAAG-3') and digested with *CfoI* restriction enzyme resulting in fragments of 241, 93 and 87 bp for the *FUT1M307<sup>G/G</sup>* genotype and fragments of 328, 241, 93, 87 bp and fragments of 328 and 93 bp for the *FUT1M307<sup>G/A</sup>* and *FUT1M307<sup>A/A</sup>* genotype, respectively.

## 2.4. In vitro villous adhesion assay

In order to determine the presence of the F18R on small intestinal villous enterocytes, an in vitro villous adhesion assay was performed as described previously (Cox and Houvenaghel, 1993; Verdonck et al., 2002). After euthanasia of the pigs, a 20 cm intestinal segment was excised from the mid jejunum of the pig and rinsed three times with icecold PBS. Subsequently, the gut segment was opened and incubated with Krebs–Henseleit buffer (160 mM, pH 7.4) with 1% (v/v) formaldehyde for 5 min at 4 °C. Next, the villi were gently scraped from the mucosae with a glass slide and stored in this buffer. Before the villi are used in the adhesion test, they were washed three times with Krebs–Henseleit buffer without formaldehyde and subsequently suspended in PBS supplemented with 1% (w/v) D-mannose to prevent the adhesion of *E. coli* by means of type 1 pili. Next, 4 × 10<sup>8</sup> bacteria of the F18 positive reference strain (F107/86) were added to an average of 50 villi in a total volume of 500 µl PBS with 1% D-mannose, followed by incubation at room temperature for 1 h while being gently shaken. Villi were examined by phase-contrast microscopy at a magnification of 600× and the number of bacteria adhering along 50 µm brush border was quantitatively evaluated by counting the number of adhering bacteria at 20 randomly selected places after which the mean bacterial adhesion was calculated. Adhesion of less than 5, between 5 and 30 and more than 30 bacteria per 250 µm brush border length was noted as negative, weak or strong positive, respectively.

## 2.5. Immunohistochemistry

After euthanasia, the abdomen was opened and a small segment of the jejunum was excised and flushed three times with icecold PBS. Subsequently, the gut segment was embedded in 2% (w/v) methocel (Fluka, Bornem, Belgium) in water, immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Thin sections ( $10\text{ }\mu\text{m}$ ) of the frozen tissue were cut and mounted on 3-aminopropyltriethoxysilane (APES, Sigma–Aldrich) coated glass slides. After drying for 3 h at  $60^{\circ}\text{C}$ , the cryosections were fixed in acetone at  $-20^{\circ}\text{C}$  for 10 min and stored at  $-80^{\circ}\text{C}$ .

The cryosections were air-dried for 1 h at room temperature, followed by washing in PBS for 5 min and incubation with 10% (v/v) goat serum in PBS for 30 min at  $37^{\circ}\text{C}$ . Subsequently, the cryosections were sequentially incubated with the primary antibodies (anti-H-2 mAb ( $13\text{ }\mu\text{g/ml}$ ; clone 92FR-A2; DAKO), anti-A-2 mAb ( $59\text{ }\mu\text{g/ml}$ ; clone 29.1; Sigma–Aldrich) or anti-CD174 ( $1/7.5$  diluted; clone A70-C/C8; Serotec)) diluted in PBS and with FITC-conjugated secondary anti-mouse antibody during 1 h at  $37^{\circ}\text{C}$ . PI-staining was performed to visualize cell nuclei. The sections were mounted in glycerol with 0.223 M 1,4-diazobicyclo(2,2,2)-octane (DABCO; Sigma–Aldrich) to prevent photobleaching and analyzed with a fluorescence microscope. To distinguish between the different degrees of expression, a semi-quantitative scoring system was established, with ‘+’ score referring to expression of blood group antigens on goblet cells only, while ‘++’ score was given when the brush border was partly covered by blood group antigens and ‘+++’ was used for fully covered brush borders.

Presence of HBGAs on gut epithelial cells was double-checked by performing the immunohistochemical staining on isolated villi of the respective animals.

## 2.6. Statistical analysis

Statistical analysis was performed using the software package SPSS Version 12.0. The correlation between all different parameters was estimated by the Spearman’s correlation test. Statistical significance was defined at  $P < 0.05$ .

## 3. Results

### 3.1. Age-dependent expression of the F18R

In the present study, the F18R is genotypically and phenotypically characterized in 74 pigs with an age between 0 and 23 weeks.

Using the RFLP-test to determine the F18R genotype (Meijerink et al., 1997), it was found that 41 and 27 of the examined pigs had the *FUTIM307<sup>GLG</sup>* and *FUTIM307<sup>GLA</sup>* genotype, respectively, both corresponding with sensitivity for F18<sup>+</sup> *E. coli* infections (Frydendahl et al., 2003). Only six pigs had the *FUTIM307<sup>ALA</sup>* genotype corresponding with resistance for F18<sup>+</sup> *E. coli*. The frequencies of the A and G alleles were 0.26 and 0.74, respectively.

The F18R expression on porcine intestinal epithelial cells was determined using an in vitro villous adhesion assay (Table 1). In the pigs that were euthanized the first few hours after birth, no adhesion was found of F18<sup>+</sup> *E. coli* to the villous epithelial cells, whereas one out of four 1.5 weeks old-piglets showed weak adhesion (13.75 adhering F18<sup>+</sup> *E. coli* per 250  $\mu\text{m}$  villus), indicating weak F18R expression. At the age of 3 weeks, 7 out of 12 genetically susceptible pigs (58.3%) showed F18R expression with 5 pigs classified as weak and 2 as strong F18R positive. In the period after weaning until 23 weeks of age, 40 out of 49 genetically susceptible pigs (81.6%) showed weak or strong F18R expression. After weaning, no changes in degree of F18R expression were found since genetically susceptible pigs aged between 4 and 12 weeks showed an average of 22.7 bacteria per 250  $\mu\text{m}$  villus and pigs older than 12 weeks showed 24.6 bacteria per 250  $\mu\text{m}$  villus.

When analyzing the correlation of the *FUTIM307* genotype to F18<sup>+</sup> *E. coli* adhesion, only pigs older than 4 weeks were taken into account to avoid age-dependent lack of F18R expression. It was found that three out of five of the more than 4 weeks old genetically resistant pigs (*FUTIM307<sup>ALA</sup>* genotype) were subdivided in the F18R<sup>−</sup> category, while 2 of the genetically F18R<sup>−</sup> pigs showed weak F18R expression with, respectively, 6.2 and 26.5 adhering bacteria per 250  $\mu\text{m}$  villus (counted in triplicate). To examine the correlation between the *FUTIM307<sup>ALA</sup>* genotype and the F18R phenotype more extensively, pigs of some local farms were screened for the *FUTIM307<sup>ALA</sup>*

genotype using the RFLP assay. Six *FUTIM307*<sup>A/A</sup> pigs were identified and included in this study (Table 2). These six genetically resistant pigs showed the F18R<sup>−</sup> phenotype.

For the genetically susceptible *FUTIM307*<sup>G/L</sup> pigs, it was found that 40 out of 49 animals showed F18R expression, while the other 9 pigs were classified as F18R<sup>−</sup>, as examined with the villous adhesion assay. No differences in F18R expression could be observed between the 'F18R positive genotypes' *FUTIM307*<sup>G/L</sup> and *FUTIM307*<sup>G/A</sup> with an average of 22.2 and 26.1 adhering bacteria per 250  $\mu$ m villus, respectively. This indicates that the single substitution of the G nucleotide to an A nucleotide on position 307 of *FUT1* has no major effect on the expression level of the F18R. The Spearman correlation coefficient showed a weak significant correlation ( $r = 0.307$ ,  $P < 0.05$ ) between the *FUTIM307* genotype and the F18R phenotype, whereas, by means of a negative control, no correlation was found with the F4R phenotype ( $r = -0.129$ ).

### 3.2. Presence of histo-blood group antigens correlates positively with F18<sup>+</sup> *E. coli* adhesion

It was suggested before that H-2 histo-blood group antigens (H-2 HBGAs) are involved in F18<sup>+</sup> *E. coli* adhesion by Snoeck et al. (2004). In the porcine AO blood group system, the H-2 sugar, which corresponds to what is termed blood group O, can be further transformed in the A-2 blood group antigen or the Le<sup>y</sup> antigen (Meijerink et al., 2001; Sepp et al., 1997) (Fig. 1). In the present study, no Le<sup>y</sup> antigens were found on the porcine epithelium using the anti-human CD174 mAb, but expression of A-2 HBGA could be

detected. To examine whether both the histo-blood group antigens H-2 and A-2 play a role in F18<sup>+</sup> *E. coli* adhesion, first of all, the correlation between the expression of both HBGAs and F18<sup>+</sup> *E. coli* adherence was investigated.

The presence of H-2 and A-2 HBGAs on the intestinal epithelial cells of pigs was determined by an immunohistochemical staining on cryosections using blood group H-2 (clone 92FR-A2) and A-2 specific antibodies (clone 29.1) (Fig. 2). Thirty pigs were found to express H-2 HBGAs on the intestinal epithelial cells, 25 pigs were A-2 positive and 19 pigs were blood group AO negative (Table 1). No pigs were observed to be positive for both the H-2 and A-2 HBGAs, indicating that in A-2 positive animals, the precursor H-2 antigen is masked for detection with the H-2 specific monoclonal antibody because of the presence of the terminal GalNAc.

For pigs with HBGA expression in the intestine, it was observed that expression was gradually increasing during the first 3 weeks of life and that expression remained in general high in older pigs. Two of the four piglets that had been euthanized a few hours after birth, were found to express the A blood group antigen very weakly (score '+') on the intestinal epithelial cells, whereas two out of four 10 days old piglets showed weak H-2 expression (score '++') and 3 out of 12 of the 3 weeks old piglets were found to be strong H-2 or A-2 positive (score '+++'). From the age of 4 weeks until the pigs were 23 weeks old, most of the pigs (64.8%) showed strong expression of the H-2 or A-2 HBGA.

To correlate the presence of HBGAs with the *FUTIM307* genotype, again, pigs from 4 weeks and older were taken into account. Four out of five pigs

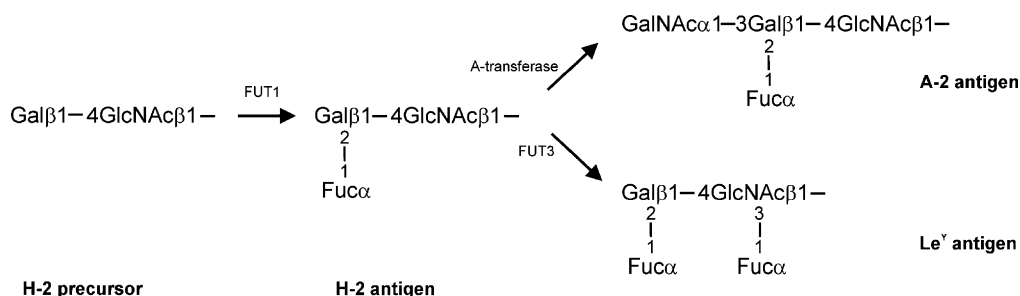


Fig. 1. Schematic representation of the biosynthetic pathway for synthesis of blood group AO related determinants from type 2 chain precursors. Gal = galactose, GlcNAc = *N*-acetylglucosamine, Fuc = fucose, GalNAc = *N*-acetylglactosamine, FUT1 = fucosyltransferase 1, FUT3 = fucosyltransferase 3.



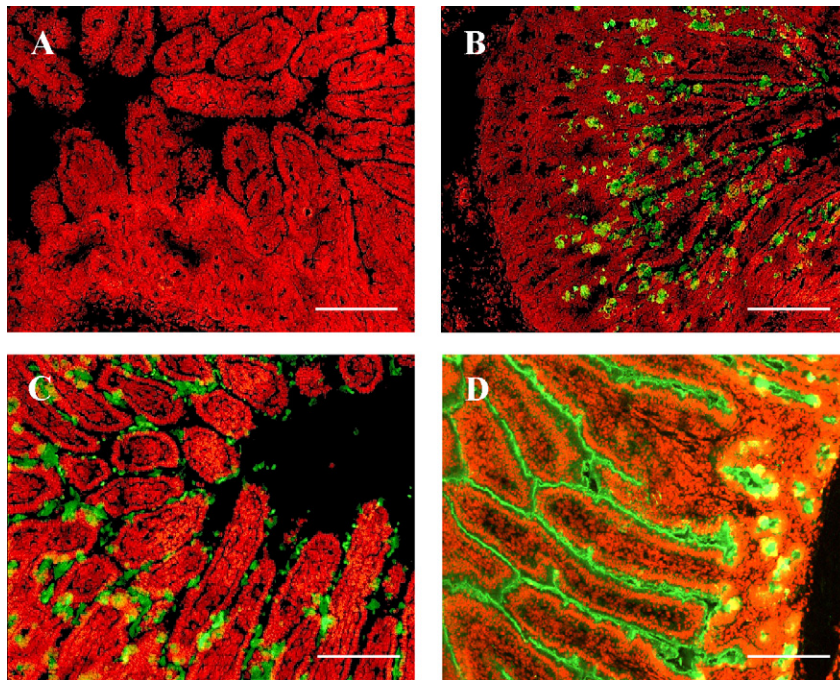


Fig. 2. Cryosections of the jejunum of four pigs of different ages with different levels of histo-blood group antigen expression. Immunohistochemical detection of blood group A-2 antigens (green fluorescence) with a specific mAb (clone 29.1; Sigma–Aldrich). Nuclei of pig gut cells are stained with propidium iodide (red fluorescence). (A) No expression of histo-blood group antigen A-2 on gut epithelial cells (score ‘–’). (B) Very weak expression of the A-2 antigen (score ‘+’). (C and D) Weak expression (score ‘++’) and strong expression of blood group antigen A-2 (score ‘+++’), respectively. Bar, 200  $\mu$ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

with the *FUT1M307<sup>A/A</sup>* genotype were found to be HBGA negative, while the other *FUT1M307<sup>A/A</sup>* pig showed very weak A-2 antigen expression. The six extra *FUT1M307<sup>A/A</sup>* pigs incorporated in this study (Table 2) also showed no H-2 or A-2 HBGA expression. This indicates that the G→A transition on bp 307 of both alleles of the *FUT1* gene influences the HBGA expression negatively. Ten out of 49 of the *FUT1M307<sup>G/L</sup>* pigs were found to be HBGA negative, whereas four pigs showed weak HBGA expression and 35 pigs had strong expression of HBGA on the intestinal epithelial cells. Using the Spearman correlation coefficient, it was found that expression of both the H-2 and A-2 HBGA was weakly correlated with the *FUT1M307* genotype ( $r = 0.45$ ,  $P < 0.01$ ).

When examining the correlation between the expression of H-2 and A-2 HBGA and F18<sup>+</sup> *E. coli* adherence (Figs. 3 and 4), it was found that the majority (13 out of 19) of the HBGA negative pigs

(score ‘–’) were classified in the F18R<sup>–</sup> category as determined by the villous adhesion test. However, villi of 5 HBGA negative pigs showed weak F18<sup>+</sup> *E. coli* adhesion (between 6.2 and 29.4 bacteria/250  $\mu$ m) and villi of 1 HBGA negative pig were found to be strong F18R<sup>+</sup> (35 bacteria/250  $\mu$ m), suggesting that additional molecules other than H-2 and A-2 HBGA might be involved in adhesion of the F18<sup>+</sup> *E. coli* strain F107/86. On the other hand, most (17 out of 19) of the H-2 positive pigs with score ‘+++’ can be found in the F18R positive categories, whereas 2 H-2 positive pigs belonged to the F18R<sup>–</sup> category. Furthermore, all pigs expressing A-2 blood group antigens (score ‘+++’) on the intestinal epithelial cells were found to be F18R<sup>+</sup>. The Spearman correlation coefficient revealed a moderate correlation between expression of the H-2 antigen and F18<sup>+</sup> *E. coli* adhesion ( $r = 0.568$ ,  $P < 0.01$ ) and between expression of the A-2 antigen and F18<sup>+</sup> *E. coli* adhesion ( $r = 0.775$ ,  $P < 0.01$ ). This correlation is not absolute

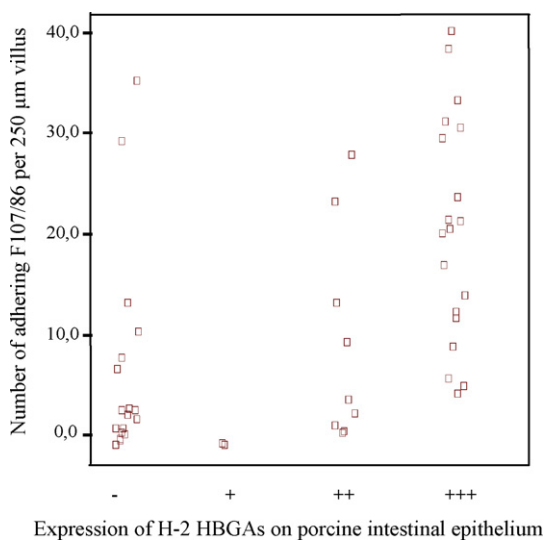


Fig. 3. Correlation between expression of H-2 histo-blood group antigens and F18<sup>+</sup> *E. coli* adhesion to porcine intestinal epithelial cells.

as there are a few pigs expressing HBGA showing no F18<sup>+</sup> *E. coli* adhesion and some HBGA negative pigs showing F18<sup>+</sup> *E. coli* adhesion. Nevertheless, it was found that all pigs with strong A-2 expression and the majority of the pigs with H-2 expression were found to be F18R positive. Expression of A-2 antigens and F18<sup>+</sup> *E. coli* adhesion showed the highest correlation.

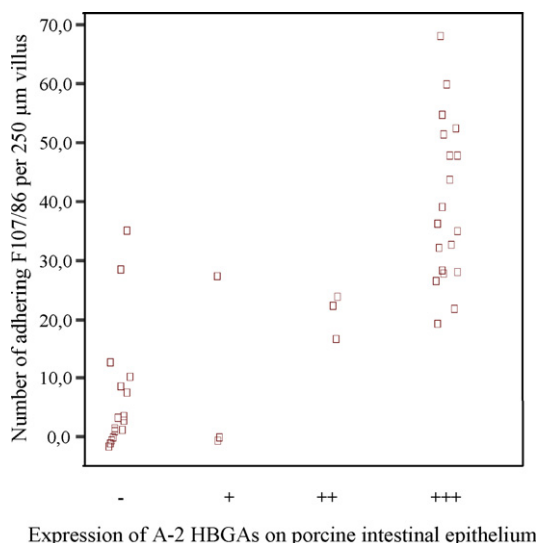


Fig. 4. Correlation between expression of A-2 histo-blood group antigens and F18<sup>+</sup> *E. coli* adhesion to porcine intestinal epithelial cells.

As a control, the correlation between expression of HBGA and F4R expression was calculated and found not to be significant (Spearman's  $r = 0.01$ ) reflecting the equal distribution of the HBGA positive and negative pigs among the three F4<sup>+</sup> *E. coli* adhesion categories.

#### 4. Discussion

F18<sup>+</sup> *E. coli* infections causing post-weaning diarrhoea and oedema disease in young pigs occur mostly 1–2 weeks post-weaning (Bertschinger et al., 1990) and lead to considerable economic losses in pig farms. Susceptibility to these F18<sup>+</sup> *E. coli* infections in pigs is shown to be dependent on the presence of the F18R on the porcine intestinal epithelial cells (Bertschinger et al., 1993; Frydendahl et al., 2003). To learn more about the F18R expression of pigs along various ages, 74 pigs ranging from 0 to 23 weeks old were tested for F18R expression using an in vitro villous adhesion test. In the present study, F18R expression is found in 10 days old piglets and appeared to increase gradually with age in the suckling phase. Despite the presence of the F18R, there is a low prevalence of F18<sup>+</sup> *E. coli* infections in suckling piglets. This suggests the importance of inhibiting molecules present in sow's milk. Indeed, it has been shown that antibodies in sow's milk provide protection to piglets against enteropathogens such as F4<sup>+</sup> and F18<sup>+</sup> *E. coli* (Deprez et al., 1986; Riising et al., 2005). In addition, there are numerous reports of molecules other than antibodies such as oligosaccharides and glycoproteins, that are able to protect against *E. coli* infections (Nollet et al., 1999; Martin et al., 2002; Morrow et al., 2004; Newburg et al., 2004; Shahriar et al., 2005).

Especially in the period of 1–2 weeks after weaning, pigs are highly susceptible to F18<sup>+</sup> *E. coli* infections and the highest number of oedema disease outbreaks is reported. This is in accordance with the strong F18R expression observed in pigs of this age. This high F18R expression level was still found in 23 weeks old pigs, the oldest age group tested in this study. This explains the occurrence of oedema disease in adult pigs (Bürge et al., 1992). Nevertheless, it is known that susceptibility to F18<sup>+</sup> *E. coli* infections decreases as the pigs become older. This decrease in

susceptibility to F18<sup>+</sup> *E. coli* infections was not correlated with loss of F18R expression, as was observed before for F5<sup>+</sup> *E. coli* (Runnels et al., 1980). One possible explanation for the decreased susceptibility could be the release of receptors in the mucus as was observed for the 987P receptors (Dean, 1990), although this is not investigated yet. In addition, low prevalence of oedema disease in these older animals could be due to immunity acquired after F18<sup>+</sup> *E. coli* infection (Verdonck et al., 2002), since it was observed that F18<sup>+</sup> *E. coli* is ubiquitously present in Flemish pig farms (Verdonck et al., 2003). Thirdly, when taken into account that oedema disease is the result of the action of the verotoxin VT2e, it must be noted that age-related changes in expression of the VT2e-receptor (Gb4) might affect the susceptibility to F18<sup>+</sup> *E. coli* disease, as was suggested for GM1, the LT-receptor (Grange et al., 2006).

The carbohydrate structure of the F18R is not characterized yet, although Snoeck et al. (2004) found inhibiting effects of the blood group antigen H-2 trisaccharide and the monoclonal H-2-specific antibody on F18<sup>+</sup> *E. coli* adhesion, suggesting a role for the H-2 sugar in F18<sup>+</sup> *E. coli* adherence. It has been shown earlier that carbohydrate structures of blood group antigens can be involved in adhesion of certain pathogenic organisms such as Norwalk virus (Marionneau et al., 2002), Noroviruses (Huang et al., 2003), *Campylobacter jejuni* (Ruiz-Palacios et al., 2003), *Helicobacter pylori* (Borén et al., 1993) to the host tissue.

Since the H-2 sugar is further transformed in the A-2 blood group antigen in genetically predisposed animals, the possible role of the A-2 tetrasaccharide in F18<sup>+</sup> *E. coli* adhesion was investigated by assessing the correlation between presence of both H-2 and A-2 HBGAs on porcine intestinal villi and F18<sup>+</sup> *E. coli* adherence. The results show that both H-2 and A-2 HBGAs are significantly positively correlated with F18<sup>+</sup> *E. coli* adhesion, although the correlation was not absolute. Five out of 19 pigs that were classified as negative for H-2 or A-2 HBGAs on the gut epithelium were found to be F18R positive. Therefore, the possibility that additional or other factors or molecules might be involved in F18<sup>+</sup> *E. coli* adhesion or that the correlation is merely a result of a genetic linkage between the F18R genes and the *FUT* loci cannot be ruled out. Furthermore, *FUT1* is an intermediate step

in the biosynthesis of HBGAs with multiple potential end products including A-2 and Le<sup>Y</sup> as shown in Figure 1. However, preliminary studies showed that F18<sup>+</sup> *E. coli* adherence could be inhibited (up to 94%) when pre-incubating the villi with 100 µg/ml of the A-2-specific monoclonal antibody (Coddens, unpublished results). This evidence rather suggests a functional role of the A-2 HBGAs in F18<sup>+</sup> *E. coli* adherence, although further studies have to be carried out to confirm this hypothesis. Furthermore, it was observed that the correlation coefficient calculated for A-2 expression and F18<sup>+</sup> *E. coli* adhesion ( $r = 0.775$ ,  $P < 0.01$ ) was higher than the one for the H-2 antigen ( $r = 0.568$ ,  $P < 0.01$ ). It might be possible that addition of an extra sugar to the precursor chain affects the affinity of the binding. This was observed for the F17a-G adhesin of F17 fimbriated *E. coli* involved in diarrhoea and septicemia in ruminants, where the GlcNAc oligomers chitobiose, chitotriose and chitotetraose showed a slightly higher affinity for the F17a-G compared to the single GlcNAc sugar (Buts et al., 2003).

When correlating the *FUT1M307* genotype to the F18R phenotype, it was found that 1 of the 12 genetically resistant pigs showed weak F18<sup>+</sup> *E. coli* adhesion and that some of the genetically susceptible pigs were F18R<sup>-</sup>. The latter could be due to additional mutations or regulatory mechanisms that affect transcription or translation of the *FUT1* gene or could be due to absence of type 2 precursor. In addition, assay limitations could contribute to the lack of a perfect correlation, since quantification of F18R expression in the villous adhesion test is arbitrary. The correlation of H-2 and A-2 HBGA expression with the *FUT1M307* genotype is in accordance with the findings of Meijerink et al. (2001) showing significant differences in activity of the  $\alpha(1,2)$ fucosyltransferase in pigs with a different *FUT1M307* genotype. The positive correlation between type 2 HBGA expression and the *FUT1M307* genotype also confirms that pig *FUT1* preferentially fucosylates type 2 chains (Cohney et al., 1999).

In conclusion, it was found that both the F18R and the HBGAs are expressed in an age-related manner. Furthermore, this study suggests that, apart from H-2 HBGAs, A-2 HBGAs might be involved in F18<sup>+</sup> *E. coli* adherence, although further studies will be necessary to confirm the possible functional role of



H-2 and A-2 HBGAs in F18<sup>+</sup> *E. coli* adherence. Characterization of the carbohydrate structure of the F18R would lead to a better understanding of the interaction between bacterial adhesins and their host cell receptors. In addition, receptor-based treatments against F18<sup>+</sup> *E. coli*-induced disease could provide a good alternative for the prophylactic or therapeutic use of antibiotics in pig food.

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