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

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- 21 antigens
- 22

23 Abstract

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25 F18⁺ Escherichia coli have the ability to colonize the gut and cause oedema disease or post-26 weaning diarrhoea by adhering to specific F18 receptors (F18R) on the porcine epithelium. 27 Although it is well established that a DNA polymorphism on base pair 307 of the *FUT1* gene, 28 encoding an $\alpha(1,2)$ fucosyltransferase, accounts for the F18R phenotype, the F18R nature is 29 not elucidated yet. The aim of the present study was to investigate the correlation between the 30 presence of H-2 histo-blood group antigens (HBGAs) or its derivative A-2 HBGAs on the 31 porcine gut epithelium and F18⁺ E. coli adherence. A significant positive correlation was 32 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⁺ E. coli adherence after examination of 74 pigs aged from zero to 23 weeks. 33 The majority of the genetically resistant pigs ($FUT1M307^{A/A}$) showed no HBGA expression 34 (91.7%) and no $F18^+$ E. coli adherence (83.3%). In addition, it was found that F18R 35 36 expression levels rise with increasing age during the first three weeks after birth and that F18R expression is maintained in older pigs (3 to 23 weeks old). Taken together, these data 37 suggest that, apart from H-2 HBGAs, A-2 HBGAs might be involved in F18⁺ E. coli 38 39 adherence.

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42 **1. Introduction**

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Newly weaned pigs are very susceptible to F18 positive *Escherichia coli* (F18⁺ *E. coli*) 44 45 infections leading to post-weaning diarrhoea or oedema disease. In this infection process, the 46 F18 receptor (F18R) plays a crucial role by mediating the binding of F18 fimbriated bacteria 47 to the intestinal epithelium. This leads to colonization of the gut and subsequent secretion of 48 entero- or verotoxins. The population of pigs consists of F18R negative (F18R) and F18R positive (F18R⁺) animals and only the latter are subject to infection with F18⁺ E. coli 49 50 (Frydendahl et al., 2003). The F18R status of pigs is genetically determined (Bertschinger et al., 1993) and susceptibility to F18⁺ E. coli infections appeared to be dependent on the activity 51 of the *FUT1* gene, encoding the alpha(1,2)-fucosyltransferase (FUT1) (Meijerink et al., 1997; 52 2000). Sequencing of the FUT1 gene of pigs resistant to F18⁺ E. coli infection revealed a 53 54 transition (G \rightarrow A) of both alleles on bp 307. This results in an amino acid substitution at position 103 (Ala -> Thr) in the resistant $FUT1M307^{A/A}$ pigs in contrast with the susceptible 55 $FUT1M307^{G/A}$ or $FUT1M307^{G/G}$ pigs. 56

Alpha(1,2)-fucosyltransferases (FUTs) are key enzymes involved in the formation of blood 57 58 group antigens of the porcine AO blood group system, which corresponds to the human ABO 59 blood group system (Sako et al., 1990). FUT transfers a fucose to H precursors 60 $(Gal\beta 1, 3/4GlcNAc\beta 1-R)$ leading to the synthesis of H blood group antigens 61 $(Gal\beta_{1,3/4}(Fuc\alpha_{1,2})GlcNAc\beta_{1-R})$. Expression of A blood group antigens ((GalNAc\alpha_{1,3})-62 $Gal\beta_{1,3/4}(Fuc\alpha_{1,2})GlcNAc\beta_{1-R})$ is due to the action of an N-acetylgalactosaminyl-63 transferase that mediates the addition of GalNAc to the H antigen. As in humans, the 64 phenotypic appearance of the A blood group antigens in pigs is dependent on the expression 65 of its precursor, the H antigen. Expression of blood group antigens is not confined to 66 erythrocytes (Landsteiner K, 1900), as they are also present on the cell surfaces of endothelial

- and a variety of epithelial cells even as in secretions (Ravn et al., 2000; Marionneau et al.,
 2001). Therefore, they are termed 'histo-blood group antigens' (HBGAs) (Clausen and
 Hakomori, 1989).
- 70 It has been shown earlier that H type 2 (H-2) (Gal β 1,4(Fuc α 1,2)GlcNAc β 1-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⁺ E. coli adhesion (Snoeck et al., 2004), however, the role of
- A-2 antigens in $F18^+ E$. *coli* adhesion has not been investigated before.
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78 2. Materials and Methods

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- 80 2.1 Pigs
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82 In this study, seventy-four pigs of 33 different litters (Belgian Landrace x Piétrain or Dutch 83 landrace) of 4 different farms were examined and subdivided in 13 groups of different ages 84 (Table 1). Each group contains at least 3 pigs out of at least 2 different litters. The newborn 85 piglets (category zero weeks old) were deprived of colostrum and euthanized maximum 4 86 hours after birth, whereas the pigs of 1.5 and 3 weeks old received sow's milk ad libitum on 87 the farm. After purchase and transport to isolation units, they received water and Baby-star 88 pellets (NV Versele-Laga, Deinze, Belgium) ad libitum until they were euthanized one or two 89 days later. From the age of 4 weeks, the pigs were routinely weaned and fed ad libitum (Pigo-90 star, NV Versele-Laga, Deinze, Belgium). In addition, six extra pigs (Belgian Landrace x 91 Piétrain), genetically resistant to $F18^+ E$. coli infections, were selected out of 2 different litters 92 and analyzed for F18R expression (Table 2). Euthanasia was performed by intravenous 93 injection of an overdose of pentobarbital (24 mg/kg; Nembutal; Sanofi Santé Animale, 94 Brussels, Belgium). The experimental and animal management procedures were undertaken in 95 accordance with the requirements of the animal care and ethics committee of the Faculty of 96 Veterinary Medicine (EC2004/32).

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98 2.2 Bacterial inoculum

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100 The verotoxigenic F18 positive reference strain 107/86 (serotype O139:K12:H1, F18ab⁺,
101 SLT-IIv⁺) was cultured on BHI agar plates (Oxoid, Basingstoke, Hampshire, England) at

- 102 37° C for a period of 18 h. The bacteria were harvested by centrifugation and suspended in 103 PBS with 1% D-mannose whereafter the concentration of the bacteria was determined by 104 measuring the optical density of the bacterial suspension at 660 nm (OD₆₆₀). An OD of 1 105 equals 10⁹ bacteria per ml, as determined by counting colony forming units.
- 106
- 107 2.3 Determination of the F18R genotype
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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'-CTTCCTGAACGTCTATCAAGA-CC-3', M307R: 5'-CTTCAGCCAGGGCTCCTTTAAG-3') and digested with *CfoI* restriction enzyme resulting in fragments of 241, 93 and 87 bp for the *FUT1M307^{G/G}* genotype and fragments of 328, 241, 93, 87 bp and fragments of 328 and 93 bp for the *FUT1M307^{G/A}* and *FUT1M307^{A/A}* genotype, respectively.

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- 117 2.4 In vitro villous adhesion assay
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119 In order to determine the presence of the F18R on small intestinal villous enterocytes, an *in* 120 vitro villous adhesion assay was performed as described previously (Cox and Houvenaghel, 121 1993; Verdonck et al., 2002). After euthanasia of the pigs, a 20 cm intestinal segment was 122 excised from the mid jejunum of the pig and rinsed three times with icecold PBS. 123 Subsequently, the gut segment was opened and incubated with Krebs-Henseleit buffer (160 124 mM, pH 7,4) with 1% (v/v) formaldehyde for 5 minutes at 4°C. Next, the villi were gently 125 scraped from the mucosae with a glass slide and stored in this buffer. Before the villi are used 126 in the adhesion test, they were washed three times with Krebs-Henseleit buffer without

127 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, $4 \ge 10^8$ bacteria of the F18 128 129 positive reference strain (F107/86) were added to an average of 50 villi in a total volume of 130 500 µl PBS with 1% D-mannose, followed by incubation at room temperature for 1 h while 131 being gently shaken. Villi were examined by phase-contrast microscopy at a magnification of 132 600 and the number of bacteria adhering along 50 µm brush border was quantitatively 133 evaluated by counting the number of adhering bacteria at 20 randomly selected places after 134 which the mean bacterial adhesion was calculated. Adhesion of less than 5, between 5 and 30 135 and more than 30 bacteria per 250 µm brush border length was noted as negative, weak or 136 strong positive, respectively.

- 137
- 138 2.5 Immunohistochemistry
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140 After euthanasia, the abdomen was opened and a small segment of the jejunum was excised 141 and flushed three times with ice cold PBS. Subsequently, the gut segment was embedded in 2 142 % (w/v) methocel (Fluka, Bornem, Belgium) in water, immediately frozen in liquid nitrogen 143 and stored at -80°C. Thin sections (10 μ m) of the frozen tissue were cut and mounted on 3-144 aminopropyl-triethoxysilane (APES, Sigma-Aldrich) coated glass slides. After drying for 3 h 145 at 60°C, the cryosections were fixed in acetone at -20°C for 10 minutes and stored at -80°C. 146 The cryosections were air-dried for 1 h at room temperature, followed by washing in PBS for 147 5 minutes and incubation with 10% (v/v) goat serum in PBS for 30 minutes at 37° C. 148 Subsequently, the cryosections were sequentially incubated with the primary antibodies (anti-149 H-2 mAb (13µg/ml; clone 92FR-A2; DAKO), anti-A-2 mAb (59µg/ml; clone 29.1; Sigma-150 Aldrich) or anti-CD174 (1/7.5 diluted; clone A70-C/C8; Serotec)) diluted in PBS and with 151 FITC-conjugated secondary anti-mouse antibody during 1 h at 37°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.

159 Presence of HBGAs on gut epithelial cells was double-checked by performing the160 immunohistochemical staining on isolated villi of the respective animals.

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162 2.6 Statistical analysis

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164 Statistical analysis was performed using the software package SPSS version 12.0. The

165 correlation between all different parameters was estimated by the Spearman's correlation test.

166 Statistical significance was defined at P < 0.05.

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168 **3. Results**

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- 170 3.1 Age-dependent expression of the F18R
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In the present study, the F18R is genotypically and phenotypically characterized in 74 pigswith an age between 0 and 23 weeks.

174 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 FUT1M307^{G/G} and FUT1M307^{G/A} genotype

176 respectively, both corresponding with sensitivity for F18⁺ E. coli in fections (Frydendahl et al.,

177 2003). Only 6 pigs had the $FUT1M307^{A/A}$ genotype corresponding with resistance for F18⁺ E.

178 *coli*. The frequencies of the A and G alleles were 0.26 and 0.74 respectively.

179 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 180 181 birth, no adhesion was found of F18⁺ E. coli to the villous epithelial cells, whereas one out of four 1.5-week-old-piglets showed weak adhesion (13.75 adhering $F18^+ E$. coli per 250 µm 182 183 villus), indicating weak F18R expression. At the age of three weeks, 7 out of 12 genetically 184 susceptible pigs (58.3%) showed F18R expression with five pigs classified as weak and two 185 as strong F18R positive. In the period after weaning until 23 weeks of age, 40 out of 49 186 genetically susceptible pigs (81.6%) showed weak or strong F18R expression. After weaning, 187 no changes in degree of F18R expression were found since genetically susceptible pigs aged 188 between 4 to 12 weeks showed an average of 22.7 bacteria per 250 µm villus and pigs older 189 than 12 weeks 24.6 bacteria per 250 µm villus.

When analyzing the correlation of the FUT1M307 genotype to F18⁺ *E. coli* adhesion, only pigs older than 4 weeks were taken into account to avoid age-dependent lack of F18R

192 expression. It was found that 3 out of 5 of the more than 4-week-old genetically resistant pigs ($FUT1M307^{A/A}$ genotype) were subdivided in the F18R⁻ category, while two of the genetically 193 194 F18R⁻ pigs showed weak F18R expression with respectively 6.2 and 26.5 adhering bacteria 195 per 250 µm villus (counted in triplicate). To examine the correlation between the $FUT1M307^{A/A}$ genotype and the F18R phenotype more extensively, pigs of some local farms 196 were screened for the $FUT1M307^{A/A}$ genotype using the RFLP assay. Six $FUT1M307^{A/A}$ pigs 197 198 were identified and included in this study (Table 2). These 6 genetically resistant pigs showed 199 the F18R⁻ phenotype.

For the genetically susceptible FUT1M307^{G/,} pigs, it was found that 40 out of 49 animals 200 201 showed F18R expression, while the other 9 pigs were classified as F18R, as examined with 202 the villous adhesion assay. No differences in F18R expression could be observed between the 'F18R positive genotypes' FUT1M307^{G/G} and FUT1M307^{G/A} with an average of 22.2 and 26.1 203 204 adhering bacteria per 250 um villus, respectively. This indicates that the single substitution of 205 the G nucleotide to an A nucleotide on position 307 of FUT1 has no major effect on the 206 expression level of the F18R. The Spearman correlation coefficient showed a weak significant 207 correlation (r = 0.307, P < 0.05) between the FUT1 M307 genotype and the F18R phenotype, whereas, by means of a negative control, no correlation was found with the F4R phenotype (r 208 209 = -0.129).

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3.2 Presence of histo-blood group antigens correlates positively with F18⁺ E. coli adhesion

It was suggested before that H-2 histo-blood group antigens (H-2 HBGAs) are involved in F18⁺ *E. coli* adhesion by Snoeck and colleagues (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^Y antigen (Meijerink et al., 2001; Sepp et al., 1997) (Figure 1). In the present study, no Le^Y 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⁺ *E. coli* adhesion, first of all, the correlation between the expression of both HBGAs and F18⁺ *E. coli* adherence is investigated.

223 The presence of H-2 and A-2 HBGAs on the intestinal epithelial cells of pigs was determined 224 by an immunohistochemical staining on cryosections using blood group H-2 (clone 92FR-A2) 225 and A-2 specific antibodies (clone 29.1) (Figure 2). Thirty pigs were found to express H-2 226 HBGAs on the intestinal epithelial cells, 25 pigs were A-2 positive and 19 pigs were blood 227 group AO negative (Table 1). No pigs were observed to be positive for both the H-2 and A-2 228 HBGAs, indicating that in A-2 positive animals, the precursor H-2 antigen is masked for 229 detection with the H-2 specific monoclonal antibody because of the presence of the terminal 230 GalNAc.

231 For pigs with HBGA expression in the intestine, it was observed that expression was 232 gradually increasing during the first three weeks of life and that expression remained in 233 general high in older pigs. Two of the 4 piglets that had been euthanized a few hours after 234 birth, were found to express the A blood group antigen very weakly (score '+') on the 235 intestinal epithelial cells, whereas 2 out of four 10-day-old piglets showed weak H-2 236 expression (score '++') and 3 out of 12 of the 3-week-old piglets were found to be strong H-2 237 or A-2 positive (score '+++'). From the age of four weeks until the pigs were 23 weeks old, 238 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 *FUT1M307* genotype, again, pigs from 4 weeks and older were taken into account. Four out of 5 pigs with the *FUT1M307^{A/A}* genotype were found to be HBGA negative, while the other *FUT1M307^{A/A}* pig showed very weak A-2

antigen expression. The 6 extra $FUT1M307^{A/A}$ 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^{G/}* pigs were found to be HBGA negative, whereas four pigs showed weak HBGA expression and 35 pigs had strong expression of HBGAs on the intestinal epithelial cells. Using the Spearman correlation coefficient, it was found that expression of both the H-2

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and A-2 HBGAs was weakly correlated with the *FUT1M307* genotype (r = 0.45, P < 0.01).

249 When examining the correlation between the expression of H-2 and A-2 HBGAs and F18⁺ E. 250 *coli* adherence (Figure 3 and 4), it was found that the majority (13 out of 19) of the HBGA 251 negative pigs (score '-') were classified in the F18R⁻ category as determined by the villous 252 adhesion test. However, villi of 5 HBGA negative pigs showed weak F18⁺ E. coli adhesion 253 (between 6.2 and 29.4 bacteria/250 µm) and villi of 1 HBGA negative pig were found to be 254 strong F18R⁺ (35 bacteria/250 μ m), suggesting that additional molecules other than H-2 and A-2 HBGAs might be involved in adhesion of the F18⁺ E. coli strain F107/86. On the other 255 256 hand, most (17 out of 19) of the H-2 positive pigs with score '+++' can be found in the F18R 257 positive categories, whereas 2 H-2 positive pigs belonged to the F18R⁻ category. Furthermore, all pigs expressing A-2 blood group antigens (score ++++) on the intestinal epithelial cells 258 259 were found to be F18R⁺. The Spearman correlation coefficient revealed a moderate 260 correlation between expression of the H-2 antigen and F18⁺ E. coli adhesion (r = 0.568, P < 261 0.01) and between expression of the A-2 antigen and F18⁺ E. coli adhesion (r = 0.775, P < 262 (0.01). This correlation is not absolute as there are a few pigs expressing HBGAs showing no $F18^+$ E. coli adhesion and some HBGA negative pigs showing $F18^+$ E. coli adhesion. 263 264 Nevertheless, it was found that all pigs with strong A-2 expression and the majority of the 265 pigs with H-2 expression were found to be F18R positive. Expression of A-2 antigens and $F18^+ E. \ coli$ adhesion showed the highest correlation. 266

As a control, the correlation between expression of HBGAs 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⁺ *E. coli* adhesion categories.

271 **Discussion**

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F18⁺ E. coli infections causing post-weaning diarrhoea and oedema disease in young pigs 273 274 occur mostly 1 to 2 weaks post-weaning (Bertschinger et al., 1990) and lead to considerable 275 economic losses in pig farms. Susceptibility to these $F18^+ E$. coli infections in pigs is shown 276 to be dependent on the presence of the F18R on the porcine intestinal epithelial cells 277 (Bertschinger et al., 1993; Frydendahl et al., 2003). To learn more about the F18R expression 278 of pigs along various ages, 74 pigs ranging from zero to 23 weeks old were tested for F18R 279 expression using an *in vitro* villous adhesion test. In the present study, F18R expression is 280 found in 10-day-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 $F18^+$ E. coli infections in 281 282 suckling piglets. This suggests the importance of inhibiting molecules present in sow's milk. 283 Indeed, it has been shown that antibodies in sow's milk provide protection to piglets against 284 enteropathogens such as F4⁺ and F18⁺ E. coli (Deprez et al., 1986; Riising et al., 2005). In 285 addition, there are numerous reports of molecules other than antibodies such as 286 oligosaccharides and glycoproteins, that are able to protect against E. coli infections (Nollet et 287 al., 1999; Martin et al., 2002; Morrow et al. 2004; Newburg et al., 2004; Shahriar et al., 288 2005).

Especially in the period of 1 to 2 weeks after weaning, pigs are highly susceptible to F18⁺ *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-week-old pigs, the oldest age group tested in this study. This explains the occurrence of oedema disease in adult pigs (Bürgi et al., 1992). Nevertheless, it is known that susceptibility to F18⁺ *E. coli* infections decreases as the pigs become older. This decrease in susceptibility to F18⁺ *E. coli* infections was not correlated

296 with loss of F18R expression, as was observed before for $F5^+$ *E. coli* (Runnels et al., 1980). 297 One possible explanation for the decreased susceptibility could be the release of receptors in 298 the mucus as was observed for the 987P receptors (Dean, 1990), although this is not 299 investigated vet. In addition, low prevalence of oedema disease in these older animals could 300 be due to immunity acquired after F18⁺ E. coli infection (Verdonck et al, 2002), since it was observed that F18⁺ E. coli is ubiquitously present in Flemish pig farms (Verdonck et al., 301 302 2003). Thirdly, when taken into account that oedema disease is the result of the action of the 303 verotoxin VT2e, it must be noted that age-related changes in expression of the VT2e-receptor (Gb4) might affect the susceptibility to $F18^+ E$. coli disease, as was suggested for GM1, the 304 305 LT-receptor (Grange et al., 2006).

The carbohydrate structure of the F18R is not characterized yet, although Snoeck and colleagues (2004) found inhibiting effects of the blood group antigen H-2 trisaccharide and the monoclonal H-2-specific antibody on F18⁺ *E. coli* adhesion, suggesting a role for the H-2 sugar in F18⁺ *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), Norovirusses (Huang et al., 2003), *Campylobacter jejuni* (Ruiz-Palacios et al., 2003), *Helicobacter pylori* (Borén et al., 1993) to the host tissue.

313 Since the H-2 sugar is further transformed in the A-2 blood group antigen in genetically 314 predisposed animals, the possible role of the A-2 tetrasaccharide in F18⁺ E. coli adhesion was 315 investigated by assessing the correlation between presence of both H-2 and A-2 HBGAs on 316 porcine intestinal villi and F18⁺ E. coli adherence. The results show that both H-2 and A-2 317 HBGAs are significantly positively correlated with $F18^+$ E. coli adhesion, although the 318 correlation was not absolute. Five out of 19 pigs that were classified as negative for H-2 or A-319 2 HBGAs on the gut epithelium were found to be F18R positive. Therefore, the possibility 320 that additional or other factors or molecules might be involved in F18⁺ E. coli adhesion or that

321 the correlation is merely a result of a genetic linkage between the F18R genes and the FUT322 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^{Y} as shown in Figure 1. 323 However, preliminary studies showed that $F18^+ E$. *coli* adherence could be inhibited (up to 324 325 94%) when pre-incubating the villi with 100 μ g/ml of the A-2-specific monoclonal antibody 326 (Coddens A., unpublished results). This evidence rather suggests a functional role of the A-2 327 HBGAs in F18⁺ E. coli adherence, although further studies have to be carried out to confirm 328 this hypothesis. Furthermore, it was observed that the correlation coefficient calculated for A-329 2 expression and F18⁺ E. coli adhesion (r = 0.775, P < 0.01) was higher than the one for the 330 H-2 antigen (r = 0.568, P < 0.01). It might be possible that addition of an extra sugar to the 331 precursor chain affects the affinity of the binding. This was observed for the F17a-G adhesin 332 of F17 fimbriated E. coli involved in diarrhoea and septicemia in ruminants, where the 333 GlcNAc oligomers chitobiose, chitotriose and chitotetraose showed a slightly higher affinity 334 for the F17a-G compared to the single GlcNAc sugar (Buts et al., 2003).

335 When correlating the FUT1M307 genotype to the F18R phenotype, it was found that one of 336 the 12 genetically resistant pigs showed weak $F18^+$ E. coli adhesion and that some of the 337 genetically susceptible pigs were F18R. The latter could be due to additional mutations or 338 regulatory mechanisms that affect transcription or translation of the FUT1 gene or could be 339 due to absence of type 2 precursor. In addition, assay limitations could contribute to the lack 340 of a perfect correlation, since quantification of F18R expression in the villous adhesion test is 341 arbitrary. The correlation of H-2 and A-2 HBGA expression with the FUT1M307 genotype is 342 in accordance with the findings of Meijerink and colleagues (2001) showing significant 343 differences in activity of the $\alpha(1,2)$ fucosyltransferase in pigs with a different FUT1M307 344 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).

347 In conclusion, it was found that both the F18R and the HBGAs are expressed in an age-348 related manner. Furthermore, this study suggests that, apart from H-2 HBGAs, A-2 HBGAs might be involved in F18⁺ E. coli adherence, although further studies will be necessary to 349 confirm the possible functional role of H-2 and A-2 HBGAs in F18⁺ E. coli adherence. 350 351 Characterization of the carbohydrate structure of the F18R would lead to a better 352 understanding of the interaction between bacterial adhesins and their host cell receptors. In 353 addition, receptor-based treatments against F18⁺ E. coli-induced disease could provide a good 354 alternative for the prophylactic or therapeutic use of antibiotics in pig food.

355

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357

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



Figure 2: Cryosections of the jejunum of 4 pigs of different ages with different levels of histoblood 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 propidiumiodide (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 μ m



Figure 3: Correlation between expression of H-2 histo-blood group antigens and F18⁺ *E. coli* adhesion to porcine intestinal epithelial cells



Expression of A-2 HBGAs on porcine intestinal epithelium

Figure 4: Correlation between expression of A-2 histo-blood group antigens and F18⁺ *E. coli* adhesion to porcine intestinal epithelial cells

Table 1: Summary of data on the age-dependent expression of the F18R and HBGAs. Abbreviations: A = adenine, G = guanine, *FUT1M307*: basepair 307 of the *FUT1* gene

age	number of pigs	FU	TIM	F18 <i>1M307</i> adher 250µ			'18 ⁺ <i>E. coli</i> herence per 50μm villus			HBGA expression						
weeks		G/G	G/A	A/A	< 5	5-30	>30		Н-2 ро	sitive			A-2 p	ositive		
					-	+	++	-	+	++	+++		+	++	+++	
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 to 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 to 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 to 18	4	2	2				4	4							4	
22 to 23	3	2	1		1		2	1			2	3				

Table 2: Overview of data on F18⁺ *E. coli* adhesion and blood group antigen expression of 12 pigs with the $FUT1M307^{4/A}$ genotype. Pigs that were included later are marked with an asterisk.

pig	age	F18 ⁺ <i>E. coli</i> adherence	HBGA expression	
	(weeks)	per 250µm villus		
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	-	