Asian-Aust. J. Anim. Sci.
Vol. 21, No. 4 : 489 - 493
April 2008

www.ajas.info

Identification and Screening of Gene(s) Related to Susceptibility to Enterotoxigenic *Escherichia coli* F4ab/ac in Piglets

Hejun Li, Yuhua Li, Xiaotian Qiu, Xiaoyan Niu, Yang Liu and Qin Zhang*

State Key Laboratory for Agrobiotechnology, Key Laboratory of Animal Genetics and Breeding of the Ministry of Agriculture, College of Animal Science and Technology, China Agricultural University, Beijing 100094, China

ABSTRACT : In 2004, Jørgensen and coworkers proposed the *MUC4* gene as a candidate gene of enterotoxigenic *Escherichia coli* (ETEC) F4ab/ac receptor in piglets and a mutation of G→C in intron 7 of *MUC4* was identified to be associated with the ETEC F4ab/ac adhesion phenotypes. In this study, we used 310 piglets of three breeds (Landrace, Large White and Chinese Songliao Black) to analyze the relationship between this mutation and the F4ab/ac adhesion phenotype. The results show that the genotypes at this site and the ETEC F4ab/ac adhesion phenotypes were not completely consistent, although they are very strongly associated. Among the individuals with genotype CC, which was identified as a resistant genotype to F4ab/ac adhesion, only 72.1% (124/172) were non-adhesive to ETEC F4ab and 77.9% (134/172) were non-adhesive to ETEC F4ac infections. This suggests that this mutation may not be the causative mutation for ETEC F4ab/ac adhesion, rather, the actual causative mutation may be in another gene closely linked to *MUC4*, or at aother site within the *MUC4* gene. Our results also suggest that the receptors of F4ab and F4ac may be determined by two different but closely linked loci. In order to screen other genes related to F4ab/ac adhesion in piglets, the mRNA profiles from six full sib piglets, of which three were adhesive to ETEC F4ab/ac and three non-adhesive, were analyzed by suppression subtractive hybridization (SSH). One up-regulated gene, *Ep-CAM*, was selected for further analysis based on its role in the intestinal epithelial cells adhesion. Using real-time RT-PCR, we found that the *Ep-CAM* gene was significantly up-regulated in the piglets adhesive to F4ab/ac. It was mapped to SSC3q11-q14 by radiation hybrid mapping. (**Key Words :** ETEC F4ab/ac, Susceptibility, Receptor, *Muc4*, *Ep-CAM*, Piglets)

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) expressing fimbriae F4 (formerly K88) is the major pathogenic bacteria causing diarrhoea and death in neonatal and post-weaning piglets (Wilson et al., 1986; Cheng et al., 2006; Wang et al., 2006). Three F4 antigenic variants including F4ab, F4ac and F4ad have been identified (Mooi et al., 1978; Guinee et al., 1979). These fimbriae mediate the adhesion of ETEC F4 to F4 variant specific receptors (Bijlsma et al., 1982) and facilitate their colonization in the small intestine by preventing intestinal peristalsis from removing the ETEC (Isaacson et al., 1988). The colonized bacteria produce enterotoxins, a heat-stable and/or heat-labile toxin, which induce massive fluid and electrolytes into the gut lumen and result in diarrhea (Moon et al., 1999).

The loci encoding for the porcine intestinal receptors for ETEC F4ab/ac were assigned to chromosome 13 by linkage analysis (Guérin et al., 1993; Edfors-Lilja et al., 1995) and refined to SSC13q41 by two research groups (Python et al., 2002; Jørgensen et al., 2003). Recently, the F4ac receptor locus was precisely located to a region flanked by SW207 and SW0283 on 13q41 (Joller et al., 2006) and our research group confirmed it and found that the locus was closely linked to SW0283 (Li, 2006). Within this region, Jørgensen et al. (2004) investigated a candidate gene *MUC4* and found that a mutation of G→C in its intron 7 was strongly associated with ETEC F4ab/ac adhesion phenotypes. The CC genotype was supposed to be associated with non-adhesive phenotype and hence inferred as resistant genotype, the GG and GC genotypes were associated with adhesive phenotype and hence inferred as susceptible genotypes. However, whether the existence of F4abR (F4ab receptor) and F4acR (F4ac receptor) is under the control of one locus or two closely linked loci is still unclear. Evidences for two loci closely linked were reported by Bonneau et al. (1990) and Guérin et al. (1993). But the results of Python et al. (2002) were in favor of a single locus.

* Corresponding Author: Qin Zhang. Tel: +86-10-62732634, Fax: +86-10-62732634, E-mail: Qzhang@cau.edu.cn
Received August 18, 2007; Accepted November 25, 2007

In this study, we analyzed the relationship between the mutation of G→C in intron 7 of the *MUC4* gene and the adhesion phenotypes of ETEC F4ab/ac in a large experiment population in order to verify the association between them found by Jørgensen et al. (2004) and to clarify whether the existence of F4abR and F4acR is determined by a single locus or two closely linked loci. Furthermore, to further reveal the genetic mechanisms of the adhesion of ETEC F4ab/ac, we used the technique of suppression subtractive hybridization to screen the genes related to the adhesion of ETEC F4ab/ac.

MATERIALS AND METHODS

Pigs

The experimental herd for this study was bred at the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. A two-generation population was used, comprised of 310 piglets and 77 parents from three different breeds, Landrace, Large White and Chinese Songliao Black. All piglets were weaned at 28 days of age, and slaughtered a week after weaning and their jejunal segments free of contents were collected for adhesion test and RNA isolation.

Adhesion test

The phenotypes (adhesion, weak-adhesion and non-adhesion) were established by means of a microscopic enterocyte adhesion test. Three *E. coli* strains included 195 (O8: K87: F4ab), 200 (O149: K91: F4ac) and 238 (O38: K99: F4-negative) from China Institute of Veterinary Drug Control in Beijing were used. Preparation of enterocytes and adhesion test were carried out using the procedure described by Baker et al. (1997) with slight modifications described by Li et al. (2007). For each piglet, 20 or more epithelial cells from its epithelial cell specimen were checked. A single epithelial cell was considered adhesive when more than five bacteria adhering to its brush border membrane. A pig was classified as adhesive if at least 10% of the checked epithelial cells were judged as adhesive (Vögeli et al., 1996), as weak-adhesive if less than 10% of the epithelial cells were judged as adhesive, yet more than 10% bind one to four bacteria, or as non-adhesive if no epithelial cells bound bacteria.

MUC4 gene test

The *MUC4* gene test was performed based on the *Xba*I-polymorphism in intron 7 of the porcine *MUC4* gene just as described by Jørgensen et al. (2004) and Jensen et al. (2006). The PCR-RFLP assay was performed and the 367 bp product was obtained from pig genomic DNA. The resistant allele (C) was indigestible by *Xba*I, whereas the susceptible allele (G) was digested into 151 and 216 bp fragments.

Suppression subtractive hybridization (SSH)

The total RNAs were isolated using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions from the jejunal segment of the piglets. Tester cDNA and driver cDNA were synthesized using the pool of total RNAs from three piglets adhesive to ETEC F4ab/ac and three piglets non-adhesive to ETEC F4ab/ac, respectively. All of these six piglets were from the same litter of Large White. PolyA$^+$ RNA was purified by Oligotex mRNA mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's guidelines. SSH was performed as described in the PCR-Select cDNA Subtraction Kit (Clontech Laboratories, Palo Alto, CA, USA). The differentially expressed PCR products were inserted into pGEM-T Easy Vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* DH10B. White clones were picked and 1 μl of each bacterial LB culture was amplified by using Nested PCR Primers 1 and 2R (Clontech Laboratories, Palo Alto, CA, USA). All positive clones were directly sequenced using an ABI 3730 DNA Analyser at BGI LifeTech Company Limited in Beijing.

Sequence analysis and database search

Searches for sequence similarity were performed with the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST) against the non-redundant sequence and the pig-specific sequences (http://www.ncbi.nlm.nih.gov/genome/guide/pig/).

Real-time RT-PCR

The total RNAs of the six piglets of Large White were prepared as described above for SSH and further treated with DNase I (Promega, Madison, WI, USA) for 30 min at 37°C. RT-PCR transcripts from the six piglets were served as templates for amplification using an ABI PRISM 7900 Sequence Detection System (Applied Biosystems) and the QuantiTect SYBR Green RT-PCR kit (Qiagen). The primers used for *Ep-CAM* were as follows: forward, 5'-TCAATGCAGGGTCTACAG-3', reverse, 5'- CCTCAAA TTACGGTGTTATG-3'. And the PCR conditions were 50°C for 2 min, 95°C for 2 min and 40 cycles of 94°C for 30 s, 61.9°C for 30 s and 72°C for 20 s, followed by one cycle of 95°C for 15 s, 60°C for 20 s and 95°C for 15 s for disassociation curve analysis. The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the endogenous assay control (primer sequence: forward, 5'-TGAGCACCAGGTTGTGTC-3' and reverse, 5'-CACCACCCTGTTGCTGT-3'). Each sample was run in triplicate to ensure quantitative accuracy, and the threshold cycle numbers (C_t) were averaged. The results were calculated using $2^{-\Delta\Delta Ct}$ method (Livak et al., 2001).

Radiation hybridization mapping

Here, we report the mapping of *Ep-CAM* gene that may

Table 1. Numbers of piglets and percentages (in the parentheses) with respect to different *MUC4* genotypes and ETEC F4ab/ac adhesion phenotypes in three pig breeds

Breed	Genotype	N	F4ab			F4ac		
			Adhesive	Weak-adhesive	Non-adhesive	Adhesive	Weak-adhesive	Non-adhesive
Landrace	GG	30 (35.7)	30	0	0	30	0	0
	GC	37 (44.0)	35	1	1	33	0	4
	CC	17 (20.2)	6	1	10	3	1	13
	Total	84	71 (84.5)	2 (2.4)	11 (13.1)	66 (78.6)	1 (1.2)	17 (20.2)
Large White	GG	11 (7.4)	11	0	0	11	0	0
	GC	60 (40.3)	59	0	1	54	4	2
	CC	78 (52.3)	14	1	63	6	8	64
	Total	149	84 (56.4)	1 (0.7)	64 (43.0)	71 (47.7)	12 (8.1)	66 (44.3)
Songliao Black	GG	0 (0)	0	0	0	0	0	0
	GC	0 (0)	0	0	0	0	0	0
	CC	77 (100)	20	6	51	6	14	57
	Total	77	20 (26.0)	6 (7.8)	51 (66.2)	6 (7.8)	14 (18.2)	57 (74.0)

Table 2. Association of *MUC4* genotypes and ETEC F4ac adhesion phenotypes

Genotype	N	F4ab		F4ac	
		Positive	Negative	Positive	Negative
GG	41	41	0	41	0
GC	97	95	2	91	6
CC	172	48	124	38	134
Total	310	184	126	170	140
x^2		158.44		167.73	
p-value		3.94E-35		3.78E-37	

be related to the process of adhesion of *E. coli* F4ab/ac. The primers (forward, 5'-TGGTCAGTGCCAGTGTACTT-3' and reverse, 5'- TCAGAGTCCTTATCGGTCCT-3') only amplified the *Ep-CAM* gene in pig, and not the hamster controls. The PCR was performed in a total volume of 20 μl using 1×PCR buffer, 200 μM of each dNTP, 0.2 mM of each primer, 1 U Taq DNA polymerase, and 25 ng of genomic DNA of the full INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel. PCR was carried out at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 59.2°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. The PCR products were analysed on 2.5% agarose gels containing ethidium bromide. Data analysis of all genes was performed through the IMpRH web site (http://www.toulouse.inra.fr/lgc/pig/RH/IMpRH.htm). The gene was typed in duplicate with the IMpRH panel.

RESULTS

Adhesion test and *MUC4* gene test

The results of ETEC F4ab/ac adhesion test and *MUC4* gene test are summarized in Table 1. There are significant differences in distributions of *MUC4* gene genotypes and F4ab/ac adhesion phenotypes in the three pig breeds. In Landrace, most of the pigs possessed the susceptible genotypes (GG or GC) and were adhesive to F4ab/ac as well. On the other hand, in Songliao Black, all of the pigs possessed the resistant genotype (CC) and most of them were non-adhesive to F4ab/ac. In Large White, about half of the pigs possessed the susceptible genotypes and were adhesive to F4ab/ac.

Associations between the *MUC4* genotypes and the F4ab/ac adhesion phenotypes

For testing the associations between the *MUC4* genotypes and the F4ab/ac adhesion phenotypes, the adhesive and weak-adhesive phenotypes were merged into one phenotype which was inferred as F4ab/ac positive. In correspondence with this, the non-adhesive phenotype was inferred as F4ab/ac negative. The associations were tested using Chi-Square test and the results were given in Table 2. It can be seen that the *MUC4* genotypes were very strongly associated with the F4ab and F4ac phenotypes (p = 3.94E-35 and 3.78E-37, respectively). All individuals with genotype GG were both F4ab and F4ac positive. 98% and 94% individuals with genotypes GC were F4ab and F4ac positive, respectively. 72% and 78% individuals with genotype CC were F4ab and F4ac negative, respectively.

Generation of SSH cDNA libraries

In order to isolate differential genes between the piglets adhesive to ETEC F4ab/ac and those non-adhesive, forward and reverse subtractive cDNA libraries were constructed. In this study, we drew our attention to the preferentially up-regulated genes in the forward subtractive cDNA library, and a total of 246 cDNA clones were obtained. After DNA

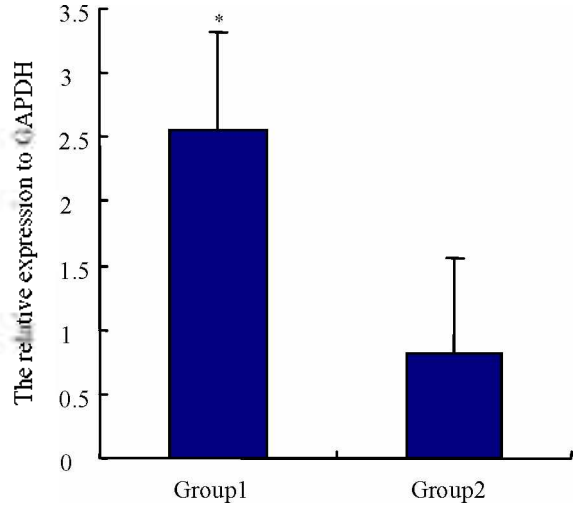

Figure 1. Relative expression of *Ep-CAM* to *GAPDH* in the intestinal epithelial cells of pigs with different phenotype. Group1: piglets adhesive to ETEC F4ab/ac; Group2: piglets non-adhesive to ETEC F4ab/ac. The difference in the expression level between the two groups is significant ($p<0.05$).

sequencing and BLAST comparison with NCBI RefSeq, GenBank and dbEST, one gene, *Ep-CAM*, was found to be involved in the process of defense against mucosal infection in the intestinal epithelial cells (Nochi et al., 2004). It was further analyzed by real-time RT-PCR and radiation hybridization mapping.

Real-time RT-PCR

The expression of *Ep-CAM* was quantified based on the C_T values obtained for the total RNA transcripts of the intestinals of the six piglets. The expression profiles of *Ep-CAM* were significantly different ($p<0.05$) between the piglets adhesive to ETEC F4ab/ac and those non-adhesive using Student's t-test (Figure 1).

Radiation hybridization mapping

Based on a combination of the IMpRH results and the cytogenetic position of the flanking markers, *Ep-CAM* was located to SSC3q11-q14 closely linked to microsatellite SW236, with a LOD score of 5.89 and a distance of 66cR. This result was consistent with the known homology between HSA2p and SSC3q.

DISCUSSION

The results of this study confirm the finding of Jørgensen et al. (2004) that the mutation of G→C in intron 7 of the *MUC4* gene was strongly associated with ETEC F4ab/ac adhesion phenotypes. However, in spite of the very strong associations, the genotypes and the phenotypes were not completely consistent, particularly for genotype CC, which is considered as resistant genotype to F4ab/ac. Of the 172 CC individuals, 48 (27.9%) were adhesive to F4ab, and 38 (22.1%) were adhesive to F4ac. This result suggests that the *MUC4* gene may not be the causative gene or the mutation of G→C in intron 7 of the *MUC4* gene may not be the causative mutation responsible for the existence of F4abR/acR, rather, it might be just a marker closely linked to the actual causative mutation, either within the *MUC4* gene or in another gene.

Several previous studies on the adhesion patterns of F4ab and F4ac revealed the existence of the patterns of F4ab+/F4ac- and F4ab-/F4ac+ (+ means positive and - means negative) in different pig populations (Bonneau et al., 1990; Baker et al., 1997; Li et al., 2007). Therefore, they suggested that F4ab and F4ac should have different receptors. Erickson et al. (1992, 1994) identified two intestinal mucin-type glycoproteins (IMTGP-1, IMTGP-2) as receptors for F4ac. Grange et al. (1996) found that a 74-kDa glycoprotein (GP74) purified from pig intestinal membranes was specifically detected *in vitro* by F4ab fimbriae, while F4ac and F4ad fimbriae did not bind to GP74. These results provided strong evidence for the two receptors hypothesis. In this study, we found that although the mutation of G→C in intron 7 of the *MUC4* gene was very strongly associated to both F4ab and F4ac adhesion phenotype, the proportions of F4ab and F4ac positive individuals among the individuals with genotype CC were different (Table 3). When the weak-adhesive phenotype classified as positive, the two proportions were 27.9% and 22.1%, respectively, which were not significantly different ($p = 0.1336$). However, in consideration of the possible errors in judging the weak-adhesive phenotype, it might be more reasonable to remove the weak-adhesive individuals when comparing the proportions. In doing so, the two proportions were 21.4% and 10.3%, respectively, which were highly significantly different ($p = 0.001$). These results prompt that the F4abR and F4acR should not be controlled by the same gene (or mutation).

By suppression subtractive hybridization, we found the *Ep-CAM* gene was significantly up-regulated in the piglets adhesive to ETEC F4ab/ac. *Ep-CAM* was regarded as a physical homophilic interaction molecule between intestinal epithelial cells and intraepithelial lymphocytes at the mucosal epithelium for providing immunological barrier as a first line of defense against mucosal infection (Nochi et al., 2004). Although *Ep-CAM* was located to SSC3q11-q14, not in the mapping region of ETEC F4ab/ac receptor gene (SSC13q41), it may play an important role in the adhesion of ETEC F4ab/ac to the intestinal epithelial cells. Further investigation is needed to reveal their relationship.

ACKNOWLEDGMENTS

We are grateful to Dr Martine Yerle (INRA, Castanet-

Tolosan, France) for providing the RH panel. The study was financially supported by the National Basic Research Program of China (Grant No. 2006CB102104) and National Natural Sciences Foundation of China (Grant No. 30430500).

REFERENCES

Baker, D. R., L. O. Billey and D. H. Francis. 1997. Distribution of K88 *Escherichia coli*-adhesive and nonadhesive phenotypes among pigs of four breeds. Vet. Microbiol. 54(2):123-132.

Bijlsma, I. G., A. de Nijs, C. van der Meer and J. F. Frik. 1982. Different pig phenotypes affect adherence of *Escherichia coli* to jejunal brush borders by K88ab, K88ac and K88ad antigen. Infect. Immun. 37(3):891-894.

Bonneau, M., Y. Duval-Iflah, G. Guérin, L. Ollivier, C. Renard and X. Renjifo. 1990. Aspects génétiques et microbiologiques de la colibacillose K88 chez le porc. Annales de la Recherche Vétérinaire. 21(4):302-303.

Cheng, D., H. Sun, J. Xu and S. Gao. 2006. PCR detection of virulence factor genes in *Escherichia coli* isolates from weaned piglets with edema disease and/or diarrhea in China. Vet. Microbiol. 115(4):320-328.

Edfors-Lilja, I., U. Gustafsson, Y. Duval-Iflah, H. Ellergren, M. Johansson, R. K. Juneja, L. Marklund and L. Andersson. 1995. The porcine intestinal receptor for *Escherichia coli* K88ab, K88ac: regional localization on chromosome 13 and influence of IgG response to the K88 antigen. Anim. Genet. 26(4):237-242.

Erickson, A. K., D. R. Baker, B. T. Bosworth, T. A. Casey, D. A. Benfield and D. H. Francis. 1994. Characterization of porcine intestinal receptors for the K88ac fimbrial adhesin of *Escherichia coli* as mucin-type sialoglycoproteins. Infect. Immun. 62(12):5404-5410.

Erickson, A. K., J. A. Willgohs, S. Y. McFarland, D. A. Benfield and D. H. Francis. 1992. Identification of two porcine brush border glycoproteins that bind the K88ac adhesin of *Escherichia coli* and correlation of these glycoproteins with the adhesive phenotype. Infect. Immun. 60(3):983-988.

Grange, P. A. and M. A. Mouricout. 1996. Transferrin associated with the porcine intestinal mucosa is a receptor specific for K88ab fimbriae of *Escherichia coli*. Infect. Immun. 64(2): 606-610.

Guérin, G., Y. Duval-Iflah, M. Bonneau, M. Bertaud, P. Guillaume and L. Ollivier. 1993. Evidence for linkage between K88ab, K88ac intestinal receptors to *Escherichia coli* and transferrin loci in pigs. Anim. Genet. 24(5):393-396.

Guinee, P. A. and W. H. Jansen. 1979. Behavior of *Escherichia coli* K antigens K88ab, K88ac, and K88ad in immunoelectrophoresis, double diffusion, and hemagglutination. Infect. Immun. 23(3):700-705.

Isaacson, R. E. 1988. Molecular and genetic basis of adherence of enteric *Escherichia coli* in animals (Ed. J. A. Roth). pp. 28-44. Virulence mechanisms of bacterial pathogens. American Society for Microbiology, Washington, DC.

Jensen, G. M., K. Frydendahl, O. Svendsen, C. B. Jørgensen, S. Cirera, M. Fredholm, J. P. Nielsen and K. Moller. 2006. Experimental infection with *Escherichia coli* O149: F4ac in weaned piglets. Vet. Microbiol. 115(1-3):243-249.

Joller, D., C. B. Jørgensen and H. U. Bertschinger. 2006. Refined linkage mapping of the *Escherichia coli* F4ac receptor gene on pig chromosome 13. Proceedings of the 30th International Conference on Animal Genetics. Brazil.

Jørgensen, C. B., S. Cirera, S. I. Anderson, A. L. Archibald, T. Raudsepp, B. Chowdhary, I. Edfors-Lilja, L. Andersson and M. Fredholm. 2003. Linkage and comparative mapping of the locus controlling susceptibility towards *E. coli* F4ab/ac diarrhoea in pigs. Cytogenet. Genome. Res. 102(1-4):157-162.

Jørgensen, C. B., S. Cirera, A. L. Archibald, L. Anderson, M. Fredholm and I. Edfors-Lilja. 2004. Porcine polymorphisms and methods for detecting them. International application published under the patent cooperation treaty (PCT). PCT/DK2003/000807 or WO2004/048606-A2.

Li, Y. 2006. Fine mapping of the intestinal receptor locus for enterotoxigenic *Escherichia coli* F4 in porcine. Ph.D. Dissertation, China Agricultural University.

Li, Y., X. Qiu, H. Li and Q. Zhang. 2007. Adhesive patterns of *Escherichia coli* F4 in piglets of three breeds. J. Genet. Genomics. 34(7):591-599.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25(4):402-408.

Mooi, F. R. and F. K. de Graaf. 1978. Isolation and characterization of K88 antigen. Fems Microbiol. Lett. 5(1): 17-20.

Moon, H. W., L. J. Hoffman, N. A. Cornick, S. L. Booher and B. T. Bosworth. 1999. Prevalences of some virulence genes among *Escherichia coli* isolates from swine presented to a diagnostic laboratory in Iowa. J. Vet. Diagn. Invest. 11(6):557-560.

Nochi, T., Y. Yuki, K. Terahara, A. Hino, J. Kunisawa, M. N. Kweon, T. Yamaguchi and H. Kiyono. 2004. Biological role of *Ep-CAM* in the physical interaction between epithelial cells and lymphocytes in intestinal epithelium. Clin. Immunol. 113(3):326-339.

Python, P., H. Jörg, S. Neuenschwander, C. Hagger, C. Stricker, E. Bürgi, H. U. Bertschinger, G. Stranzinger and P. Vögeli. 2002. Fine-mapping of the intestinal receptor locus for enterotoxigenic *Escherichia coli* F4ac on porcine chromosome 13. Anim. Genet. 33(6):441-447.

Vögeli, P., H. U. Bertschinger, M. Stamm, C. Stricker, C. Hagger, R. Fries, J. Rapacz and G. Stranzinger. 1996. Genes specifying receptors for F18 fimbriated *Escherichia coli*, causing oedema disease and postweaning diarrhoea in pigs, map to chromosome 6. Anim. Genet. 27(5):321-328.

Wang, J., S. W. Jiang, X. H. Chen, Z. L. Liu and J. Peng. 2006. Prevalence of fimbrial antigen (K88 variants, K99 and 987P) of enterotoxigenic *Escherichia coli* from neonatal and post-weaning piglets with diarrhea in central China. Asian-Aust. J. Anim. Sci. 19(9):1342-1346.

Wilson, R. A. and D. H. Francis. 1986. Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. Am. J. Vet. Res. 47(2):213-217.