Animal (2014), **8:6**, pp 859–866 © The Animal Consortium 2014
doi:10.1017/S1751731114000779

animal

Inheritance of porcine receptors for enterotoxigenic *Escherichia coli* with fimbriae F4ad and their relation to other F4 receptors

A. Rampoldi[1], H. U. Bertschinger[1], E. Bürgi[2], G. Dolf[3], X. Sidler[2], A. Bratus[1], P. Vögeli[1] and S. Neuenschwander[1†]

[1]*Institute of Agricultural Sciences, ETH Zurich, 8092 Zurich, Switzerland;* [2]*Department of Farm Animals, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland;* [3]*Institute of Genetics, Vetsuisse Faculty, University of Berne, 3012 Berne, Switzerland*

(Received 7 November 2013; Accepted 27 February 2014; First published online 11 April 2014)

Enteric Escherichia coli *infections are a highly relevant cause of disease and death in young pigs. Breeding genetically resistant pigs is an economical and sustainable method of prevention. Resistant pigs are protected against colonization of the intestine through the absence of receptors for the bacterial fimbriae, which mediate adhesion to the intestinal surface. The present work aimed at elucidation of the mode of inheritance of the F4ad receptor which according to former investigations appeared quite confusing. Intestines of 489 pigs of an experimental herd were examined by a microscopic adhesion test modified in such a manner that four small intestinal sites instead of one were tested for adhesion of the fimbrial variant F4ad. Segregation analysis revealed that the mixed inheritance model explained our data best. The heritability of the F4ad phenotype was estimated to be 0.7 ± 0.1. There are no relations to the strong receptors for variants F4ab and F4ac. Targeted matings allowed the discrimination between two F4ad receptors, that is, a fully adhesive receptor (F4adR^{FA}) expressed on all enterocytes and at all small intestinal sites, and a partially adhesive receptor (F4adR^{PA}) variably expressed at different sites and often leading to partial bacterial adhesion. In pigs with both F4ad receptors, the F4adR^{PA} receptor is masked by the F4adR^{FA}. The hypothesis that F4adR^{FA} must be encoded by at least two complementary or epistatic dominant genes is supported by the Hardy–Weinberg equilibrium statistics. The F4adR^{PA} receptor is inherited as a monogenetic dominant trait. A comparable partially adhesive receptor for variant F4ab (F4abR^{PA}) was also observed but the limited data did not allow a prediction of the mode of inheritance. Pigs were therefore classified into one of eight receptor phenotypes: A1 (F4abR^{FA}/F4acR^{+}/F4adR^{FA}); A2 (F4abR^{FA}/F4acR^{+}/F4adR^{PA}); B (F4abR^{FA}/F4acR^{+}/F4adR^{-}); C1 (F4abR^{PA}/F4acR^{-}/F4adR^{FA}); C2 (F4abR^{PA}/F4acR^{-}/F4adR^{PA}); D1 (F4abR^{-}/F4acR^{-}/F4adR^{FA}); D2 (F4abR^{-}/F4acR^{-}/F4adR^{PA}); E (F4abR^{-}/F4acR^{-}/F4adR^{-}).*

Keywords: enterotoxigenic *E. coli*, fully adhesive, partially adhesive, inheritance of F4ad receptors, pig

Implications

Enterotoxigenic *Escherichia (E.) coli* are a major cause of diarrhoea and death among young pigs. *Escherichia coli* infections contribute significantly to the use of antibiotics. Bacteria carrying different fimbriae are able to colonize the small intestine by adhering to specific receptors. There they produce toxins which may turn the infected pig sick. Selection for genetic resistance is a sustainable approach for improving piglet health, performance and welfare and to reduce antibiotic use. Present breeding results show that the genetic background of disease resistance to *E. coli* F4ad colonization is a complex trait and includes at least three genes.

† E-mail: stefan.neuenschwander@usys.ethz.ch

Introduction

Enterotoxigenic *Escherichia coli* diarrhoea in neonatal and weaned pigs causes severe economic losses (Melkebeek *et al.*, 2013). The disease is a frequent indication for oral use of antimicrobials (Jensen *et al.*, 2011), a practice causing severe concern due to the continuously increasing microbial resistances. Immunoprophylaxis appears principally effective. So far, however, no vaccine for protection of weaned pigs is available on the European market (Melkebeek *et al.*, 2013). At the long range, breeding of genetically resistant pigs offers a sustainable and economic alternative. Spontaneously resistant pigs due to lack of adhesion of *E. coli* to the intestinal mucosa were detected in most breeds of pigs. In porcine *E. coli* the two families of adhesive bacterial fimbriae F18 and F4 are highly predominant in weaned pigs all over the world. The two variants of the fimbriae F18 bind to the same host

receptor, whereas the three F4 variants F4ab, F4ac and F4ad each exhibit a specific binding pattern including variation in binding strength (Bijlsma *et al*., 1982; Billey *et al*., 1998). The fully adhesive receptor(s) for F4ab and F4ac is/are either controlled by one gene (*F4bcR*) or by two closely linked genes (Python *et al*., 2005; Yan *et al*., 2009; Schroyen *et al*., 2012).

The fimbrial variant F4ad has received little attention so far. This may be due to the complex observations cited above as well as to the limited prevalence of this fimbrial variant in the Western hemisphere (Guinée and Jansen, 1979). In addition, lower virulence compared with the other F4 variants was reported (Francis *et al*., 1999). In contrast, a high prevalence was observed in Central China (Wang *et al*., 2006). Chinese reports on the development of vaccines against *E. coli* F4ad with high virulence in neonatal pigs are of limited credibility since the authors and the Chinese Veterinary Culture Collection are not willing to clarify the confusion regarding the bacterial type strains they used.

The genetics of the partially adhesive (PA) receptors for F4ab and for F4ad, however, are less clear. Bijlsma and Bouw (1987) postulated a separate locus for the F4ad receptor underlying complete or partial suppression by an epistatic gene. They were struck at obtaining adhesive progeny from phenotypically resistant parents. In contrast, Hu *et al*. (1993) distinguished a high affinity F4ad receptor cosegregating with receptors for F4ab and F4ac from a low affinity F4ad receptor expressed only in pigs under 16 weeks of age. The hypothesis of two F4ad receptors was supported by earlier observations in the Swiss experimental herd. The PA receptor was found in adult pigs as well. The high standard deviations in the percentage of enterocytes expressing F4adR led to confusion and did not allow any conclusion regarding the mode of inheritance (Python *et al*., 2005).

In the following evidence is given for the existence of two F4ad receptors, their relation to other F4 variant receptors is analysed, and hypotheses are presented as to the modes of inheritance of the F4ad receptors.

Material and methods

Pigs

The animals used in this study originated from an experimental herd at the Department of Farm Animals, Vetsuisse Faculty at the University of Zurich. In 1998 a Large White purebred family and a Large White/Landrace crossbred family were originally bred for eight generations. Since the year 2000, the *E. coli* F4 microscopic adhesion test (MAT) was routinely applied to determine the inheritance of the resistant and susceptible alleles to the three variants of *E. coli* F4 fimbriae. A total of 489 pigs from 63 litters (456 offspring as well as 33 breeders including 19 founders and 14 offspring) were used to elucidate the inheritance of the receptors for *E. coli* F4ad (F4adR).

Sampling of intestinal tissue and MAT

Pigs were slaughtered and phenotyped at 7 months of age. Founders and the offspring which were used for breeding were tested when they were eliminated from breeding. Occasionally we slaughtered weaner pigs (51) at 2 to 3 months of age. Usually, feed was withheld for 16 h before the pigs were slaughtered; water and straw were always offered.

For the MAT the small intestine was separated from the mesentery, and at four sites a 10 to 20 cm empty segment of jejunum was taken. One segment was taken 2 m distal from the cranial mesenteric artery (site A); another one 2 m proximal to the ileocaecal valve (site D); and the other two segments at 1/3 (site B) and 2/3 (site C) of the distance between sites A and D. All segments were opened longitudinally, placed in PBS-EDTA, and stored at 4°C until further processing. The interval between killing and sampling of intestinal tissue was not more than 20 to 40 min to avoid degeneration of the epithelial intestinal cells. The enterocytes were prepared and the MAT performed according to Vögeli *et al*. (1996) and Python *et al*. (2002). *Escherichia coli* test strains E68I (O 141: K 85ab: F4ab), G4 (O 45: K (E 65): F4ac) and Guinée (O 8: K 87: F4ad) were procured from the Veterinary Laboratory Agency Weybridge, Surrey GB (Thorns *et al*., 1987). Expression of the fimbriae was checked serologically and the variant specificity by PCR (Alexa *et al*., 2001). Adhesive and non-adhesive enterocytes were conserved frozen and used as controls in each MAT. All four segments were tested for F4ad adhesion and sites B or C also for F4ab and F4ac. The same person performed sampling of jejunum, preparation and classification of the enterocytes.

Twenty intact enterocytes were scored under an optical microscope; an enterocyte was classified as adhesive if more than five bacteria adhered to the brush border. Twenty additional enterocytes were scored if adhesion of more than five bacteria was observed in >0% and <30% of the scored enterocytes. In the case of F4ad pigs with 0% of adhesive enterocytes at all the four sites were considered to be resistant (R). Based on the phenotyping of complete families for F4ad adherence at four sites of the small intestine, we propose the term fully adhesive (FA) for the phenotype exhibiting more than 85% adhesive enterocytes at all sites. For pigs with more than 0% and <85% adhesive enterocytes in at least one of the sites, we propose the term partially adhesive (PA) (Table 1). We postulate a similar classification for F4abR but have insufficient evidence.

Biopsies

In order to perform matings with known F4ad phenotypes eight pigs (four founders and three breeders, one pig was not used for breeding) were selected, and a biopsy was taken from an undeterminable site of the small intestine under general anaesthesia at around 1 month of age. The biopsies were repeated around 5 months later. The results of the biopsies were completed at slaughter by the MAT examination at intestinal sites A through D (Table 2).

Segregation analysis and estimation of heritability

A total of 489 pigs split into three pedigrees were included in the segregation analysis. To each pig one of the three F4ad

Table 1 *Criteria for determination of the F4 phenotypes based on the results of the microscopic adhesion test (MAT)*

Phenotype	Criteria[1]
Resistant (R)	0% adhesive enterocytes at intestinal sites A through D[2]
F4ab FA	>85% adhesive enterocytes (site B or C)[2]
F4ab PA	F4ac resistant, F4ab susceptible (>0% adhesive enterocytes[2])
F4ac	>85% adhesive enterocytes (site B or C)[2]
F4ad FA	>85% adhesive enterocytes at intestinal sites A through D[2]
F4ad PA	>0% and <85% adhesive enterocytes in at least one of the four intestinal sites A through D[2]

FA = fully adhesive; PA = partially adhesive.
[1]A total of 20 to 40 enterocytes were tested per site A through D.
[2]Adhesive enterocyte: > 5 adherent bacteria per enterocyte.

Table 2 *Determination of the F4ad phenotype (per cent adhesive enterocytes) by two subsequent biopsies at an age of 1 month (biopsy 1), 6 months (biopsy 2) and at slaughter (sites A through D)*

		Biopsy		MAT at slaughter				
Pig	Sex	1	2	A	B	C	D	F4adR[1]
2570	Female	0	0	0	0	0	0	R
2577	Female	0	0	0	0	0	0	R
2554	Male	0	0	0	0	0	0	R
2557	Male	0	0	0	0	0	0	R
2528	Female	90	100	100	100	100	100	FA
2452	Male	20	100	8	60	8	95	PA
2559	Male	20	93	10	15	55	95	PA
2464	Female	0	95	0	0	3	75	PA

R = resistant; FA = fully adhesive; PA = partially adhesive.
[1]Phenotypes based on the MAT at slaughter.

phenotypes was allocated: R, FA or PA. The prevalences for R, FA and PA were 25%, 35% and 40%, respectively. The values were equal in both sexes. Likelihoods were computed with the Pedigree Analysis Package version 5.0 (Hasstedt, 2002) and maximized with NPSOL (Gill *et al*., 1984).

Five different models were considered in the analysis. A general genetic model estimating the allele frequency, the transmission probabilities, the dominance effect, and the displacement to characterize a major gene, and the heritability to characterize a polygenic component was compared with an environmental model, where a major gene was excluded by setting the transmission probabilities equal to the allele frequency and the heritability to zero. In the case where the general genetic model turns out to explain the data better than the environmental model, it is compared with a mixed inheritance model which is the very same model with the exception that the transmission probabilities are set to be Mendelian. In the case where the mixed inheritance model turns out to be better than the general genetic model it is compared with a major gene model and a polygenic model. The major gene model is the same as the mixed inheritance model with the exception that a polygenic component is excluded by setting the heritability to zero.

The heritability for F4adR was estimated using the program MTDFREML (Boldman *et al*., 1995).

In order to further elucidate the inheritance of the F4ad receptors we performed repetitive and targeted matings where the same sire or dam was mated to different phenotypes. The largest litters (>12 progeny) were analysed and the hypothetical genotypes of the parents were deduced from the phenotypes of the offspring. A χ^2 value was calculated for the Hardy–Weinberg equilibrium for each litter.

Results

Biopsies

The phenotypic results of the two biopsies were only consistent for the $F4adR^{FA}$ and the resistant phenotype (R) whereas $F4adR^{PA}$ phenotype gave misleading results when compared with the MAT results at slaughter (Table 2). We therefore decided to test four sites of the small intestine to reliably differentiate the three phenotypes.

F4-phenotyping

Only 19.6% of the investigated pigs were resistant to all *E. coli* F4 variants (Table 3). In all phenotypes $F4acR^{+}$ (average over 98% of adhesive enterocytes determined at site B or C) we also found F4ab full adhesion to the same extent (97.7%, data not shown). Among the 63 (12.9%) pigs which did not express F4acR we found in three independent pedigrees 26 pigs of the phenotype $F4acR^{-}/F4abR^{+}$ ($F4abR^{PA}$). In these pigs the numbers of F4ab adhesive enterocytes were significantly reduced (average 14.4%, range from <1% to 60%), compared with the $F4abR^{FA}/F4acR^{+}$ phenotypes (data not shown). The designations for the porcine phenotypes were derived from Bijlsma and Bouw (1987).

F4ad FA and PA receptors

From the offspring we classified 171 pigs (36.4%) as FA, 177 (37.6%) as PA, and 122 (26%) as resistant to *E. coli* F4ad adhesion (Table 4). The matings of R × R parents generated only R pigs. The matings of FA × FA pigs generated only FA pigs. However, the matings R × PA, R × FA, FA × PA and PA × PA generated FA, PA and R offspring.

Progeny of F4adR phenotyped parents

The numbers of adhesive enterocytes varied considerably within the same site except for the R × R and FA × FA matings. All other mating types resulted in more than one phenotype in the offspring and therefore in a high standard deviation of the mean numbers of adhesive enterocytes (Table 4). However, there seems to be a tendency that the numbers of adhesive enterocytes are increasing from sites A through D.

Segregation analysis and estimation of the heritability

The segregation analysis revealed that the mixed inheritance model explained our data best (Table 5), as the mixed

inheritance model compared with the general genetic model needs less parameters to explain the data. The mixed inheritance model revealed that the F4ad-adhesion is controlled by a major gene and a polygenic component. This implies that phenotypes FA and PA are not the product of a single receptor gene.

The heritability of the F4adR phenotype was estimated to be 0.7 ± 0.1 and the 95% confidence interval spans from 0.53 to 0.87.

Inheritance of F4ad receptors

In the offspring of R × PA and PA × PA matings we found FA phenotypes (Table 6). This is not explainable by a one locus model and therefore we tested a two locus model for FA and a one locus model for PA, respectively. We then assigned hypothetical genotypes for F4ad receptors to the breeder pigs and the Hardy–Weinberg equilibrium was calculated comparing the number of expected pigs with the number of observed pigs for each phenotype in each litter (Table 6).

Table 3 *Eight F4 receptor phenotypes. The presence of the receptor is marked with* ●

	F4abR[1]			F4adR[2]			
Phenotype	FA	PA	F4acR[1]	FA	PA	No. of pigs	%
A1	●		●	●	(●)[3]	155	31.7
A2	●		●		●	144	29.5
B	●		●			31	6.3
C1		●		●	(●)[3]	17	3.5
C2		●			●	9	1.8
D1				●	(●)[3]	1	0.2
D2					●	36	7.4
E						96	19.6
					Total	489	100

FA = fully adhesive (>85% adhesive enterocytes); PA = partially adhesive (>0% to 85%).
[1]For the detection of F4abR and F4acR only one intestinal segment was examined.
[2]Four segments examined for F4adR.
[3]The F4adR^{FA} phenotype masks the expression of the F4adR^{PA} phenotype.

Hardy–Weinberg equilibrium (χ^2: $P < 0.05$) was respected in all litters but one. Eight pigs (in bold) expressed unexpected phenotypes (8/232 = 3.4% from offspring presented in Table 6). These pigs were phenotyped as R and came from matings R × FA and R × PA.

Discussion

The biopsies contributed significantly to this study, because they permitted to identify resistant breeders *in vivo*. They turned out to be quite laborious. Several pigs developed inflammatory adhesions despite the great efforts taken for aseptic operation conditions.

Statistical analyses indicate that F4ad receptors are highly heritable and regulated by a major gene and a polygenic component which could not be further characterized. The fact that mating types R × PA, R × FA, FA × PA and PA × PA have generated FA, PA and R pigs shows that the FA phenotype masks the PA phenotype, and that FA and PA are independently inherited. Moreover, matings of PA × PA and of R × PA leading to FA phenotypes in the offspring support the hypothesis that FA is controlled by more than one gene. These interpretations of the F4adR inheritance are still

Table 5 *Segregation analysis of the* Escherichia coli *F4ad receptor phenotypes*

Models to compare	d.f.	−2 ln likelihood	χ^2	d.f.	*P*-value
General genetic	7	727.2	334.5	4	**<0.001**
Environmental	3	1061.7			
General genetic	7	727.2	1.8	3	0.626
Mixed inheritance	4	729.0			
Mixed inheritance	4	729.0	38.0	1	**<0.001**
Major gene	3	767.0			
Mixed inheritance	4	729.0	28.4	3	**<0.001**
Polygenic	1	757.4			

Comparison of Pedigree Analysis Package (PAP) models. The differences in the −2 ln likelihoods of the different models follow a χ^2 distribution where the degrees of freedom (d.f.) are equal to the difference in the number of parameters estimated. Significant differences ($P < 0.01$) are in bold.

Table 4 *Distribution of the three F4ad receptor phenotypes FA, PA and R in the progeny of phenotyped parents*

			% adhesive enterocytes ± s.d.[1]				Phenotypes of progeny		
Mating type	No. of matings	No. of progeny	A[2]	B[2]	C[2]	D[2]	FA	PA	R
R × R	7	45	0	0	0	0	0	0	45
R × FA	16	178	50 ± 48	52 ± 47	69 ± 39	74 ± 36	81	79	18
R × PA	15	107	8 ± 25	11 ± 27	21 ± 34	31 ± 39	6	55	46
FA × FA	11	63	99 ± 1	99 ± 2	99 ± 1	99 ± 1	63	0	0
FA × PA	5	36	43 ± 49	44 ± 47	50 ± 48	52 ± 46	12	15	9
PA × PA	9	41	31 ± 40	44 ± 44	55 ± 42	67 ± 37	9	28	4
Total	63	470					171	177	122

R = resistant; FA = fully adhesive receptor (F4adR^{FA}), PA = partially adhesive receptor (F4adR^{PA}).
[1]Adhesive enterocyte: >5 adherent bacteria per enterocyte. 20 to 40 enterocytes tested per site.
[2]Sampling sites in the small intestine.

Table 6 *Analyses of the largest litters (⩾12 pigs,* n *= 232 offspring) for demonstration of the inheritance of the F4ad receptors*

				Offspring									
	Parents			System FA		System PA		Expected			Observed	χ^2	
No. litters/no. pigs	Mating-type	Sire/dam	Hypothetical genotype	Genotype	%	Genotype	%	Phenotype	Frequency %	No.	No.	F4adR	FA
2/24	R×	2831	aa Bb ww	aa bb	50	ww	100	R	100	24	24	0.00	
	R	2842	aa bb ww	aa Bb	50			PA	0	0	0		
								FA	0	0	0		
2/27	FA×	2674	AA Bb WW	Aa bb	25	Ww	100	R	0	0	0	0.95	0.24
	R	2577	aa Bb ww	Aa B-	75			PA	25	6.8	9		
								FA	75	20.2	18		
2/31[1]	FA×	2674	AA Bb WW	Aa bb	50	Ww	100	R	0	0	**2**[1]	0.81	0.40
	R	2570	aa bb ww	Aa Bb	50			PA	50	15.5	11	If R = PA	
								FA	50	15.5	18		
1/14[2]	FA×	2674	AA Bb WW	Aa bb	50	Ww	100	R	0	0	**3**[2]	**4.57**	2.29
	R	2840	aa bb ww	Aa Bb	50			PA	50	7	8	**If R = PA**	
								FA	50	7	3		
1/13[1]	FA×	2674	AA Bb WW	Aa bb	50	Ww	100	R	0	0	**1**[1]	0.08	0.04
	R	2842	aa bb ww	Aa Bb	50			PA	50	6.5	6	If R = PA	
								FA	50	6.5	6		
1/ 15	FA×	2988	AA Bb WW	Aa bb	25	Ww	100	R	0	0	0	0.51	0.13
	R	2577	aa Bb ww	Aa B-	75			PA	25	3.8	5		
								FA	75	11.2	10		
1/13	FA×	2859	Aa Bb Ww	aa bb	25	ww	50	R	37.5	4.9	7	1.65	0.01
	R	2842	aa bb ww	aa Bb	25	Ww	50	PA	37.5	4.9	3		
				Aa bb	25			FA	25	3.2	3		
				Aa Bb	25								
2/12	R×	2831	aa Bb ww	aa B-	50	Ww	100	R	0	0	0	3.00	1.50
	FA	2791	Aa BB WW	Aa B-	50			PA	50	6	9		
								FA	50	6	3		
1/14	R×	2831	aa Bb ww	aa bb	50	ww	50	R	50	7	6	0.29	
	PA	2726	aa bb Ww	aa Bb	50	Ww	50	PA	50	7	8		
								FA	0	0	0		
2/20	R×	2831	aa Bb ww	aa bb	25	ww	50	R	37.5	7.5	11	3.47	1.80
	PA	2766	Aa bb Ww	Aa bb	25	Ww	50	PA	37.5	7.5	7		
				aa Bb	25			FA	25	5	2		
				Aa Bb	25								
1/12[1]	R×	2831	aa Bb ww	aa bb	50	Ww	100	R	0	0	**2**[1]	0.00	
	PA	2878	aa bb WW	aa Bb	50			PA	100	12	10	If R = PA	
								FA	0	0	0		
1/12	R×	2554	aa bb ww	aa bb	100	ww	50	R	50	6	7	0.33	
	PA	2726	aa bb Ww			Ww	50	PA	50	6	5		
								FA	0	0	0		

Table 6: (*Continued*)

	Parents			Offspring									
				System FA		System PA		Expected		Observed		χ^2	
No. litters/no. pigs	Mating-type	Sire/dam	Hypothetical genotype	Genotype	%	Genotype	%	Phenotype	Frequency %	No.	No.	F4adR	FA
1/13	FA ×	2732	AA Bb WW	A- B-	100	WW	100	R	0	0	0	0.00	
	FA	2791	Aa BB WW					PA	0	0	0		
								FA	100	13	13		
1/12	PA ×	2559	Aa bb WW	Aa bb	25	W-	100						
	PA	2450	aa Bb Ww	aa Bb	25			R	0	0	0		
				Aa bb	25			PA	75	9	11	1.78	1.33
				Aa Bb	25			FA	25	3	1		

The same sire or dam appears in different mating types. Offspring of repetitive matings were put together. Hypothetical genotypes of parents were deduced from phenotypes observed in progeny. For each litter, the parents, the number of piglets, the F4adR phenotypes and F4adR hypothetical genotypes of the parents and the offspring are indicated. The genotype frequencies in the systems FA and PA, the expected and the observed phenotypes are shown. A chi-square value (χ^2) for the Hardy-Weinberg equilibrium is calculated for each mating. A separate χ^2 is calculated only for the FA genotype. Significant differences ($P < 0.05$) are in bold.
[1]In these matings resistant pigs were not expected based on the parents supposed genotype. If the pigs were classified as PA, the χ^2 showed no significant difference ($P < 0.05$).
[2]In this mating resistant pigs were not expected based on the parents supposed genotype. χ^2 showed a significant difference ($P < 0.05$) even if the pigs classified as resistant were considered as PA pigs.

consistent with the above mentioned mixed inheritance model of the segregation analysis. The PA phenotype was not an artefact caused by poor cell quality or mistakes in the MAT. We observed that only pigs with no adhesive enterocytes in all sites were truly resistant. Pigs with even <1% of F4ad adhesive enterocytes were shown to produce susceptible progeny if mated with a true resistant pig (data not shown). We postulate that the FA receptor is encoded by two complementary or epistatic genes, with the alleles *A* and *a*, or *B* and *b*, respectively. It is expressed only when both hypothetical alleles, *A* and *B*, occur in the dominant form, either homozygously or heterozygously. The PA receptor is controlled by only one gene with the alleles *W* and *w*. It is expressed as a mendelian dominant trait. To test this hypothesis we performed repetitive and targeted matings in which the same pig was mated to different phenotypes (Table 6), for example, sire 2674 was mated to four different resistant dams and sire 2831 was mated to five different dams which were resistant, FA or PA. Dam 2842 was mated to R and FA sires. Although we increased the number of intestinal sites for the adhesion test to four compared with only one site used in previous studies (Python *et al.*, 2002 and 2005) it cannot be excluded that we mistyped PA pigs by chance as resistant pigs.

A separate χ^2 was calculated in litters with expected FA phenotype to test the two gene inheritance model for the FA receptor. The data showed no significant difference in all litters examined for FA (Table 6).

Hu *et al.* (1993) described a high affinity F4ad receptor co-segregating with receptors for F4ab and F4ac forming the phenotype $F4abR^+/F4acR^+/F4adR^+$ which is expressed during the entire life cycle. The low affinity receptor is associated with the adhesion phenotype $F4abR^-/F4acR^-/F4adR^+$ and its expression is terminated at 16 weeks of age. In contrast we found high affinity (FA) and low affinity (PA) F4ad receptor expression independent of the F4ab and F4ac receptor phenotype (Table 3). A further explanation for this discrepancy could be the higher threshold value for resistant phenotypes used by Hu *et al.* (1993). Moreover, we identified $F4ad^{PA}$ receptors independent of the age but in a variable number of the small intestinal sites (Table 2, pigs 2452, 2559 and 2464). Furthermore fifteen pigs of phenotypes A2, C2 and E2 ($F4ad^{PA}$) were between one and 5 years of age. In all studies so far a specimen of only one site of the small intestine was phenotyped. Therefore, there is a chance to classify partially adhesive ($F4ad^{PA}$) pigs as resistant and to postulate an age dependent expression of the F4ad receptor.

Our model for F4adR inheritance is mostly valid and confirms Bijlsma and Bouw's (1987) assumption of the existence of more than one gene controlling the F4ad receptor. Besides mistyping mistakes in allocating the hypothetical genotypes of the parents cannot be excluded. This would be the case if receptor FA expression would be controlled by more than two genes. Mutations in the genes controlling receptors for *E. coli* F4ad adhesion or expression of PA inhibitors could explain the inhomogeneous presence of the PA *E. coli* F4ad receptor in the small intestine. Pigs should be tested for the PA phenotype at

more than four intestinal sites to show the variation of the number of adhesive enterocytes. Finally, genome scans should be performed to find possible candidate genes for the hypothesized FA and PA F4ad receptor loci.

The receptor for F4ab is either controlled by one gene or by two closely linked genes together with F4ac (Python *et al.*, 2005; Yan *et al.*, 2009; Schroyen *et al.*, 2012). Ren *et al.* (2012) showed evidence that *MUC13* is a good candidate for the F4ac receptor. Among the 489 pigs tested with MAT, 26 showed a resistance to *E. coli* F4ac and a partial susceptibility to *E. coli* F4ab ($F4abR^{PA}/F4acR^{-}/F4adR^{+}$) (Table 3). Most pigs with PA F4ab receptor originated from litters with a common ancestor indicating that a genetic component may be involved in the $F4abR^{PA}/F4acR^{-}$ phenotype. *Escherichia coli* F4ab adhesion was tested in the MAT by examining only one intestinal site. In a pig examined at five sites for $F4abR^{PA}$, adhesion values varied between 0% and 87% (personal observation of A. Bratus). Therefore some of the F4ab resistant phenotypes may have the PA $F4abR^{PA}$. It is possible that like *E. coli* F4ad, *E. coli* F4ab adhesion is also governed by two receptors: $F4abR^{FA}$ and $F4abR^{PA}$ somehow associated with the $F4adR^{PA}$ phenotype. It may be speculated that *E. coli* F4ab can partially bind to a mutated *E. coli* $F4ad^{PA}$ receptor. More data are necessary to further examine this hypothesis.

In summary, we think that our data give strong evidence for the existence of eight F4 receptor phenotypes which refines the classification proposed by Bijlsma *et al.* (1982), Bijlsma and Bouw (1987) and Hu *et al.* (1993) who proposed four and five F4 receptor phenotypes. Following the notation of Bijlsma and Bouw (1987) we propose A1 ($F4abR^{FA}/F4acR^{+}/F4adR^{FA}$); A2 ($F4abR^{FA}/F4acR^{+}/F4adR^{PA}$); B ($F4abR^{FA}/F4acR^{+}/F4adR^{-}$); C1 ($F4abR^{PA}/F4acR^{-}/F4adR^{FA}$); C2 ($F4abR^{PA}/F4acR^{-}/F4adR^{PA}$); D1 ($F4abR^{-}/F4acR^{-}/F4adR^{FA}$); D2 ($F4abR^{-}/F4acR^{-}/F4adR^{PA}$); E ($F4abR^{-}/F4acR^{-}/F4adR^{-}$).

Furthermore, we propose the existence of two F4ab and two F4ad receptors, a FA and a PA receptor. The $F4abR^{FA}$ and $F4adR^{FA}$ are detected throughout the small intestine with more than 85% of adhesive enterocytes. The $F4adR^{PA}$ receptor is irregularly distributed with a tendency to higher expression in the lower small intestine.

The $F4adR^{FA}$ is presumably controlled by two genes which have an epistatic component, whereas the $F4adR^{PA}$ is controlled by a dominant gene.

To our knowledge *E. coli* F4ad adhesion susceptibility in the pig is the first example in farm animals of a complex trait including three genes and epistasis for which we were able to determine a potential mode of inheritance.

Acknowledgements

Biopsies of the intestines were performed by Dr med. vet. C.S. Schwandt, Clinics for Small Animal Surgery, Department for Small Animals, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland. General anaesthesia was performed by the Anaesthesiology Unit (Prof. Dr med. vet. R. Bettschart-Wolfensberger) of the Equine Department, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland. The study was supported by the Swiss National Science Foundation (Grant No. 3100A0-120255/1), by the ETH Zurich, and the SUISAG, Sempach (Dr Andreas Hofer)

References

Alexa P, Štouračová K, Hamřík J and Rychlík I 2001. Gene typing of the colonisation factors K88 (F4) in the enterotoxigenic *Escherichia coli* strains isolated from diarrhoeic piglets. Veterinární Medicína 46, 46–49.

Bijlsma IG and Bouw J 1987. Inheritance of K88-mediated adhesion of *Escherichia coli* to jejunal brush borders in pigs: a genetic analysis. Veterinary Research Communications 11, 509–518.

Bijlsma IG, de Nijs A, van der Meer C and Frik JF 1982. Different pig phenotypes affect adherence of *Escherichia coli* to jejunal brush borders by K88ab, K88ac, or K88ad antigen. Infection and Immunity 37, 891–894.

Billey LO, Erickson AK and Francis DH 1998. Multiple receptors on porcine intestinal epithelial cells for the three variants of *Escherichia coli* K88 fimbrial adhesin. Veterinary Microbiology 59, 203–212.

Boldman KG, Kriese LA, van Vleck LD and Kachman SD 1995. A manual for use of MTDFREML – a set of programs to obtain estimates of variances and covariances. US Department of Agriculture, Agricultural Research Service, University of Nebraska, Lincoln, NE, USA. ftp://aipl.arsusda.gov/pub/outgoing/mtdfreml/mtdfrman.pdf

Francis DH, Erickson AK and Grange PA 1999. K88 adhesins of enterotoxigenic *Escherichia coli* and their porcine enterocyte receptors. Advances in Experimental Medicine and Biology 473, 147–154.

Gill PE, Murray W, Saunders MA and Wright MH 1984. User's guide for NPSOL (version 2.1): A Fortran package for nonlinear programming. Systems Optimization Laboratory, Department of Operations Research, Stanford University, Stanford, CA, USA.

Guinée PA and Jansen WH 1979. Behavior of *Escherichia coli* K antigens K88ab, K88ac, and K88ad in immunoelectrophoresis, double diffusion, and hemagglutination. Infection and Immunity 23, 700–705.

Hasstedt SJ 2002. PAP: pedigree analysis package, version 5.0. Department of Human Genetics, University of Utah, Salt Lake City, UT, USA.

Hu ZL, Hasler-Rapacz J, Huang SC and Rapacz J 1993. Studies in swine on inheritance and variation in expression of small intestinal receptors mediating adhesion of the K88 enteropathogenic *Escherichia coli* variants. Journal of Heredity 84, 157–165.

Jensen VF, Eborg H-D and Aarestrup FM 2011. Indications and patterns of therapeutic use of antimicrobial agents in the Danish pig production from 2002 to 2008. Journal of Veterinary Pharmacology and Therapeutics 35, 33–46.

Melkebeek V, Goddeeris BM and Cox E 2013. ETEC vaccination in pigs. Veterinary Immunology and Immunopathology 152, 37–42.

Python P, Jörg H, Neuenschwander S, Hagger C, Stricker C, Bürgi E, Bertschinger HU, Stranzinger G and Vögeli P 2002. Fine-mapping of the intestinal receptor locus for enterotoxigenic *Escherichia coli* F4ac on porcine chromosome 13. Animal Genetics 33, 441–447.

Python P, Jörg H, Neuenschwander S, Asai-Coakwell M, Hagger C, Bürgi E, Bertschinger HU, Stranzinger G and Vögeli P 2005. Inheritance of the F4ab, F4ac and F4ad *E. coli* receptors in swine and examination of four candidate genes for F4acR. Journal of Animal Breeding and Genetics 122 (suppl. 1), 5–14.

Ren J, Yan X, Ai H, Zhang Z, Huang X, Ouyang J, Yang M, Yang H, Han P, Zeng W, Chen Y, Guo Y, Xiao S, Ding N and Huang L 2012. Susceptibility towards enterotoxigenic *Escherichia coli* F4ac diarrhea is governed by the *MUC13* gene in pigs. PLos One 7, e44573.

Schroyen M, Stinckens A, Verhelst R, Niewold T and Buys N 2012. The search for the gene mutations underlying enterotoxigenic *Escherichia coli* F4ab/ac susceptibility in pigs: a review. Veterinary Research 43, 70.

Thorns CJ, Boarer CDH and Morris JA 1987. Production and evaluation of monoclonal antibodies directed against the fimbrial adhesins produced by *Escherichia coli* enterotoxigenic for piglets. Research in Veterinary Science 43, 233–238.

Vögeli P, Bertschinger HU, Stamm M, Stricker C, Hagger C, Fries R, Rapacz J and Stranzinger G 1996. Genes specifying receptors for F18 fimbriated *Escherichia coli*, causing oedema disease and postweaning diarrhoea in pigs, map to chromosome 6. Animal Genetics 27, 321–328.

Wang J, Jiang SW, Chen XH, Liu ZL and Peng J 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-Australasian Journal of Animal Sciences 19, 1342–1346.

Yan X, Huang X, Ren J, Zou Z, Yang S, Ouyang J, Zeng W, Yang B, Xiao S and Huang L 2009. Distribution of *Escherichia coli* F4 adhesion phenotypes in pigs of 15 Chinese and western breeds and a White Duroc × Erhualian intercross. Journal of Medical Microbiology 58, 1112–1117.