

Note

Escherichia coli K88 adhesion : A comparison of Chinese and Large White piglets

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Summary

A comparative trial was performed on 15 *Large White* and 12 *Chinese* germ-free, colostrum-deprived piglets, maintained in plastic film isolators. Shortly after successive oral inoculations of 2 *E. coli* strains, one K88-positive and one K88-negative, the piglets were sacrificed and bacterial enumerations of the 2 strains were done on 3 different intestinal segments of each pig, using both the lumen content and the washed intestinal wall. The percentage of bacteria specifically bound to the intestinal wall was estimated, and the presence or absence of the piglet K88 intestinal receptor was thus inferred. The results tended to indicate that the 12 *Chinese* piglets lacked this receptor, as against 7 *Large White*'s. This raises the question of possible breed differences in resistance to this type of colibacillosis.

Key words : K88 adhesion, *E. coli*, pig, genetic resistance.

Résumé

*L'attachement de Escherichia coli K88 :
comparaison entre porcelets Chinois et Large White*

Un essai comparatif a été réalisé sur 15 porcelets *Large White* et 12 porcelets *Chinois*, prélevés à la naissance de manière axénique, privés de colostrum et placés en isolateur sous film plastique. Après inoculation par voie orale de 2 souches de *E. coli*, l'une possédant l'antigène d'attachement K88 (K88⁺), l'autre dépourvue de cet antigène (K88⁻), les porcelets sont sacrifiés et 3 segments différents de l'intestin grêle sont prélevés sur chaque porcelet en vue de numérations des 2 souches bactériennes, à la fois dans le contenu intestinal et sur la paroi. Le pourcentage de bactéries spécifiquement attachées à la paroi est ainsi estimé et la présence ou l'absence chez le porcelet du récepteur intestinal K88 peut en être déduite. Les résultats indiquent que les 12 porcelets *Chinois* ne possèdent pas ce récepteur et doivent donc être résistants à l'attachement de K88, contre 7 porcelets *Large White*. Cela soulève la question de l'existence possible de races résistantes à cette colibacillose.

Mots clés : Attachement de K88, *E. coli*, porcs, résistance génétique.

It is recognized that most porcine neonatal diarrhoea are due to proliferation of enterotoxigenic *E. coli* strains in the small intestine (reviewed by SOJKA, 1971). From 34 to 56 p. 100 of the *E. coli* strains isolated from diarrhoeal piglets by GUINEE & JANSEN (1979), SÖDERLIND & MÖLLBY (1979) and MOON *et al.* (1980) possessed a surface antigen called K88, which enables bacteria to adhere to receptor sites located on the intestinal epithelium (ARBUCKLE, 1970 ; JONES & RUTTER, 1972 ; BERTSCHINGER *et al.*, 1972 ; WILSON & HOHMANN, 1974). Three serological variants of this K88 antigen (i.e. K88ab, K88ac and K88ad) have been described by ØRSKOV *et al.* (1964) and GUINEE & JANSEN (1979).

The discovery of a genetic basis for resistance to K88 adhesion has aroused a considerable interest (SELLWOOD *et al.*, 1974). Genetically resistant piglets lack the intestinal receptor and the genetic basis is monofactorial, a recessive gene conferring resistance. The system has been described in detail by GIBBONS *et al.* (1977), who also offered an explanation for the persistence of the susceptibility gene in pig populations. They assumed that the heterozygous genotype might be at a disadvantage, owing to the inability of a resistant sow to produce K88 antibodies. By observing the incidence of an outbreak of diarrhoea in an experimental herd with various combinations of piglet and sow genotypes, SELLWOOD (1979) was able to afford some experimental support to the above hypothesis and, in particular, he showed that the highest incidence of diarrhoea was in the susceptible progeny of resistant dams. Recently, the existence of several adhesive pig phenotypes has been suggested by BIJLSMA *et al.* (1981), in connection with the antigenic variation of K88. In fact, these authors show the existence of a non-adhesive phenotype, resistant to the 3 serological variants, and of 4 different pig phenotypes exhibiting adhesion to at least one K88 variant. The genetic basis of this variation appears to be still unresolved.

This genetic resistance exists at variable frequencies in the pig populations so far investigated : from 9 to 90 p. 100 homozygous resistant pigs in U.K. herds (WALTERS & SELLWOOD, 1982), 60 p. 100 in a *Swedish Yorkshire* × *Landrace* stock (EDFORS-LILJA *et al.*, 1982), 12 p. 100 in a commercial herd in Australia (SNODGRASS *et al.*, 1981). But in those studies resistance was assessed by applying the *in vitro* brush border test of SELLWOOD *et al.* (1975), with no specification of the K88 variant used in the tests, though the K88ac variant was used in the original paper just mentioned. The differences observed may thus result from variations of the *E. coli* tester strains. However no breed differences appear to have been so far suggested. This note reports observations indicating that such differences could exist and that even the existence of homozygous resistant breeds is not to be excluded.

Our results were obtained while investigating interactions between several *Escherichia coli* strains in the digestive tract of germ-free, colostrum deprived piglets maintained in plastic film isolators and fed with autoclaved concentrated cow milk. These animals were orally inoculated with 10⁹ viable cells of different *E. coli* strains cultivated at 37 °C for 18 h. It was first shown that 2 day-old *Large White* piglets inoculated with an enterotoxigenic *E. coli* strain possessing the K88 antigen (strain K88⁺ Ent⁺) usually die within 2 days (DUVAL-IFLAH *et al.*, 1983). However, when the same treatment was applied to a couple of *Chinese* piglets born from the *Chinese* stock imported in France in 1979 and described by LEGAULT & CARITEZ (1983), these animals survived up to 6 days. The same survival time was obtained when 2 day-old germ-free *Large White* piglets were inoculated with an enterotoxigenic *E. coli* strain lacking the K88 antigen (Ent⁺ K88⁻). These preliminary results suggested that *Chinese* piglets might be resistant to attachment of the K88 *E. coli* strain. This prompted us to realize a comparative trial

on germ-free, colostrum deprived piglets maintained in the same conditions as those mentioned above in order to test for K88 adhesion. Twelve *Chinese* piglets, 6 from the *Meishan* breed and 6 from the *Jiaying* breed, as well as 15 *Large White* (LW) piglets were used. When they were 2 day-old, they were orally inoculated with 10^9 viable cells from an 18 h culture of a non enterotoxigenic *E. coli* strain lacking the K88 antigen (K88⁻ Ent⁻). Two days later, they were inoculated in the same way with the K88⁺ Ent⁺ strain. The animals were sacrificed 18 h later and treated as described by SMITH & LINGGOOD (1971), except for the fact that only 3 segments were removed from the small intestine: 1 from the proximal end of jejunum (S1), 1 from the jejuno-ileal junction (S2) and 1 from the distal end of ileum (S3). Bacterial numerations of the 2 associated strains were performed in the lumen content and on the washed intestinal wall using selective media as previously described (DUVAL-IFLAH *et al.*, 1982).

A number of non specifically attached bacteria (NA) is retained on the intestinal wall, due to lumen residues which cannot be fully washed off. Therefore, the ratio of attached (A) to non-attached (NA) bacteria is used as an *in vivo* adhesion test. However, A/NA cannot be directly measured, but it is estimated in the following manner, knowing the ratios of the 2 strains counted in the washed intestinal wall (P) and in the lumen content (p) respectively. Under the assumption that lumen residues remaining on the intestinal wall contain x K88⁻ bacteria and y K88⁺ bacteria in the same ratio as in the

TABLE 1

Discrimination between adherent and non-adherent intestines.

Différenciation des intestins adhérents et non-adhérents.

Breed (race)	Strains (souches)	Range ^(a) of the A/NA ratio (étendue de variation du rapport A/NA)	Adherence (adhésion)
<i>Large White</i>	K88 ⁻ Ent ⁺ and K88 ⁻ Ent ⁻	8 ^(b)	-
<i>Large White</i>	K88 ⁺ Ent ⁺ and K88 ⁻ Ent ⁻	7 ^(b) 8 ^(b)	- +
<i>Chinese</i>	K88 ⁺ Ent ⁺ and K88 ⁻ Ent ⁻	12 ^(b)	-
	Scale (échelle)	0 — 8 — 200	

(a) The range given represents the extreme limits of the 95 p. 100 confidence intervals for A/NA in the segment with the highest A/NA value in each piglet.

(L'étendue indiquée représente les limites extrêmes de l'intervalle de confiance à 95 p. 100 pour A/NA dans le segment avec la valeur A/NA la plus élevée de chaque porcelet.)

(b) Number of piglets. (Nombre de porcelets).

lumen content, the P ratio exceeds p by a quantity which only depends on the number A of K88⁺ bacteria attached to the intestinal wall, and thus $P = (x + A)/y$, whereas $p = x/y$. As the total number of non-attached bacteria, K88⁺ and K88⁻, is $NA = x + y$, it can easily be seen that $A/NA = (P - p)/(p + 1)$.

P and p can be directly evaluated from bacterial counts performed on intestinal wall and in lumen content, respectively. However, the A/NA ratio is subject to rather large sampling variation. Therefore, 8 *Large White* piglets have been inoculated as described above, except for the fact that they received the K88⁻ Ent⁺ strain instead of the K88⁺ Ent⁺ one, in order to evaluate the maximal values which may be reached by this ratio in the absence of bacterial attachment. Table 1 shows that the upper limit of the 95 p. 100 confidence interval of the A/NA ratio obtained with these *Large White* piglets never exceeds 8. Similar results were obtained with 12 *Chinese* piglets inoculated with K88⁺ Ent⁺ and K88⁻ Ent⁻ strains. These animals are thus presumed to be resistant to K88 attachment. On the contrary, in 8 *Large White* piglets associated with K88⁺ Ent⁺ and K88⁻ Ent⁻ strains, the lower limit of the A/NA confidence interval, in the segment with the highest A/NA value in each piglet, always exceeds 8, which indicates that they are susceptible to K88 attachment. Table 2 gives the mean bacterial population levels found on the intestinal walls of piglets which have just been described and the mean A/NA in all segments.

TABLE 2

Viable counts of Escherichia coli on intestinal tissues of gnotobiotic piglets.

Numérations de Escherichia coli dans les tissus intestinaux de porcelets gnotobiotiques.

Breed (Race)	Nb piglets (Nombre de porcelets)	Strains (Souches)	Mean (s.d.) of log ₁₀ E. coli ^(a) [Moyenne (e.t.) de log ₁₀ E. coli]			Mean of A/NA ratio (Moyenne du rapport A/NA)	Presumed ^(b) phenotype (Phénotype préssumé)
			S1	S2	S3		
<i>Large White</i>	8	K88 ⁻ Ent ⁺	6.9 (0.7)	6.6 (1.5)	7.3 (0.2)	1.03	Control piglets
		K88 ⁻ Ent ⁻	6.7 (0.4)	6.3 (1.0)	6.9 (0.2)		
<i>Large White</i>	8	K88 ⁺ Ent ⁺	7.8 (1.1)	8.5 (0.6)	8.5 (0.6)	29.4	Adherent
		K88 ⁻ Ent ⁻	5.2 (2.3)	6.4 (1.1)	6.2 (0.9)		
<i>Large White</i>	7	K88 ⁺ Ent ⁺	5.4 (1.9)	6.6 (1.2)	6.7 (1.5)	0.51	Non adherent
		K88 ⁻ Ent ⁻	5.2 (1.3)	6.8 (0.8)	6.9 (1.3)		
<i>Chinese</i>	12	K88 ⁺ Ent ⁺	4.9 (1.3)	4.6 (1.3)	5.5 (0.9)	0.44	Non adherent
		K88 ⁻ Ent ⁻	5.5 (1.9)	5.7 (0.9)	6.6 (0.7)		

(a) Mean number of *E. coli* per g of tissue, expressed in log₁₀ and standard deviation in brackets; S1, S2 and S3 are intestinal segments removed from the jejunal proximal end, the jejuno-ileal junction and the ileal distal end, respectively.

(Nombre moyen de *E. coli* par g de tissu, exprimé en log₁₀ et écart-type entre parenthèses; S1, S2 et S3 sont les segments prélevés respectivement à l'extrémité proximale du jéjunum, à la jonction jéjuno-iléale et à l'extrémité distale de l'iléon.)

(b) Classification explained in table 1. (Voir le tableau 1 pour la classification.)

The *E. coli* strain used in this experiment bears the K88 ab antigen and, from the previously mentioned results of BIJLSMA *et al.* (1981), the "resistant piglets" of our sample may be resistant, either to the 3 variants or to only 2 of them. Further tests are therefore needed before definite conclusions can be reached. In view of this, it would certainly be unsafe to draw firm conclusions as to the status of the 2 *Chinese* breeds investigated with respect to K88 adhesion. Further reasons to be cautious are the limited sample size and the restricted sampling within breed (only 3 sires and 9 dams in the *Large White* sample and 2 sires and 3 dams in the *Chinese* sample, this one resulting from an initial importation of only 1 male and 2 females of each breed). Our results only suggest the possibility of genetic variation between breeds for that character. However, if the above results happened to be confirmed, the implications of breed resistance to K88 diarrhoea would deserve consideration. The question could also be raised of a possible link between such a resistance and the low preweaning mortality generally observed in *Chinese* breeds and confirmed by the 3 p. 100 mortality (relative to born alive) observed in purebred litters of the 2 above-mentioned breeds by LEGAULT & CARITEZ (1983). This could be an example of a favourable gene preserved under certain farm conditions and for which selection would appear to be "relaxed" in modern pig husbandry conditions.

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