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J.-P. Chappuis, Yvonne Duval-Iflah, R. Ducluzeau, P. Raibaud, Marie-France Ouriet, P. Guillaume

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Resistance of gnotobiotic Large White and Chinese piglets to *in vivo* attachment of a K88ab enterotoxigenic *Escherichia coli* strain

J.-P. CHAPPUIS, Yvonne DUVAL-IFLAH, R. DUCLUZEAU, P. RAIBAUD

with the technical collaboration of Marie-France OURIET and P. GUILLAUME

Laboratoire d'Ecologie Microbienne, I.N.R.A., 78350 Jouy-en-Josas, France.

Summary. *In vivo* adhesion of a K88ab-positive enterotoxigenic *Escherichia coli* (ETEC) strain to the small intestinal wall of gnotobiotic, colostrum-deprived Chinese and Large White piglets was investigated. A non-enterotoxigenic, attachment factor-deprived *E. coli* strain, inoculated in association with the K88ab ETEC strain, was used as a marker to determine the content residues on the intestinal walls of gnotobiotic piglets. Both strains were selectively numerated in the luminal contents and on the washed wall from three segments of the small intestine. *In vivo* attachment was assessed by the Γ ratio between the number of K88ab ETEC adherent to the wall and the number of both the *E. coli* strains which came from the luminal content residues and were not completely removed by washing. This Γ ratio was first calculated in one group of 8 Large White piglets associated with the marker strain and with a K88ab antigen-deprived *E. coli* which derived from the parental K88ab ETEC strain. Thus, the range of the Γ ratio was determined in those piglets where no attachment was expected. A comparison between the latter values of the Γ ratio and the values obtained from 15 Large White and 10 Chinese piglets inoculated with the marker strain and the K88ab ETEC strain allowed us to classify the Large White piglets into adhesive (8 piglets) and non-adhesive (7 piglets) phenotypes and to show that the 10 Chinese piglets belonged to the non-adhesive phenotype.

Introduction.

It is recognized that most cases of porcine neonatal diarrhea are characterized by the proliferation of enterotoxigenic *Escherichia coli* in the small intestine (Sojka, 1971) ; many of these *E. coli* (34 to 56 %) bear the proteinaceous K88 antigen on their surface (Söderlind and Möllby, 1979 ; Guinee and Jansen, 1979b ; Moon *et al.*, 1980). This antigen has been identified as plasmid-mediated fimbriae which have the ability to adhere specifically to receptor sites located on the brush borders of pig enterocytes (Stirm *et al.*, 1967 ; Jones and Rutter, 1972 ; Smith and Lingood, 1971 ; Smith and Huggins, 1978 ; Sellwood, 1980). But Sellwood *et al.* (1975), using an *in vitro* brush border test,

demonstrated the existence of two phenotypes, *i.e.* those which possess the receptor, and consequently are susceptible to K88 antigen adhesion, and those which are resistant because they lack the receptor. The synthesis of this receptor has been shown to be determined by two alleles at a single locus which are inherited in a simple Mendelian way. The dominant allele is expressed as a receptor for the K88 antigen and therefore only homozygous recessive pigs are resistant to K88 adhesion (Sellwood *et al.*, 1975 ; Rutter *et al.*, 1975). Three serological variants of the K88 antigen, designated as K88ab, K88ac, and K88ad, have been identified (Ørskov *et al.*, 1964 ; Guinee and Jansen, 1979a). Recently, Bijlsma *et al.* (1981) showed the existence of five piglet phenotypes in relation with the three serological K88 antigen variants. When K88 adhesion was assessed by applying the *in vitro* brush border test of Sellwood *et al.* (1975), with no specification of the K88 serological variant, it was shown that genetic resistance to K88 adhesion might exist in variable frequencies ranging from 9 to 90 % (Walters and Sellwood, 1982 ; Edfors-Lilja *et al.*, 1982 ; Snodgrass *et al.*, 1981). However, no breed differences appear to have been suggested so far.

The aim of the present work was to assess the resistance of Chinese piglets as compared to Large White piglets, using an *in vivo* attachment test performed on germ-free piglets inoculated with the same K88-positive *E. coli* strain.

Material and methods.

Piglets. — Germ-free colostrum-deprived piglets were used. They were obtained by spontaneous delivery, immediately decontaminated as previously described (Ducluzeau *et al.*, 1976), and maintained in plastic-film isolators (La Calhène, 5, rue Emile-Zola, 95870 Bezons) where they were fed autoclaved concentrated cow's milk supplemented with 0.9 % glucose and a vitamin mixture including A_1, B_1, C, D_3, E, K_3, B_2, PP, B_5, B_6, H (« Vitamino », Deltavit, France). Twenty-three Large White piglets were obtained from an INRA experimental herd and 10 Chinese piglets from two Chinese breeds (Meishan and Jiaxing) imported into France in 1979 and described by Legault and Caritez (1982).

Bacterial strains. — The *E. coli* strains listed on table 1 have been previously described (Duval *et al.*, 1983). Strain C5148 ($K88^+Ent^+$) was an enterotoxigenic, tetracycline-resistant *E. coli* (ETEC) isolated from a diarrheic piglet by L. Renault. It harboured a K88 plasmid encoding for surface antigen K88ab and, in broth culture, produced both heat-labile (LT) and heat-stable (ST) enterotoxins. Strain C5148 ($K88^-Ent^+$) was a K88-negative derivative of the former strain which had spontaneously lost its K88 plasmid but was still tetracycline-resistant and enterotoxigenic (ETEC). The K88 plasmid present in strain C5148 ($K88^+Ent^+$) was shown to encode for both the surface antigen and the utilization of raffinose, as reported by Smith and Parsell (1975). This character was used for differential counts of the K88-positive strain and its K88-negative derivative. Strain EM0 was a non-enteropathogenic, plasmid-free *E. coli* (NETEC), isolated from a healthy human fecal flora (Duval *et al.*, 1981) and strain EM4 was a rifampicin-resistant derivative of strain EM0. The selective counting media are described on table 1.

TABLE 1

Characteristics of E. coli *strains and selective counting media.*

N° of strains	Source	Characteristics (1)	Serotype (2)	Enterotoxin produced (3)	Media used for selective counts (4)
EM0	Human fecal flora	Lac ±	02	—	Drig
EM4	EM0	Lac + ; rpoB, Su	02	—	Drig-Rif
C5148 Ent$^+$, K88$^+$	Piglet diarrhoea	Raf + ; Tc, Km, Lv ; Ent$^+$; K88 ab, 987 P$^-$	0117	LT, ST	DCA-Raf-Tc
C5148 Ent$^+$, K88$^-$	C5148 Ent$^+$, K88$^+$	Raf$^-$; Tc, Km, Lv ; Ent$^+$; K88$^-$, 987 P$^-$	0117	LT, ST	DCA-Raf-Tc

(1) Raf$^+$, raffinose fermented ; Raf$^-$, raffinose not fermented ; Lac ±, lactose slowly fermented ; rpoB, resistant to rifampin ; Su, Tc, Km, Lv, resistance to sulfonamide, tetracycline, kanamycin, lividomycin ; Ent$^+$, enterotoxigenic ; K88, 987 P, adhesion factors tested by Institut Mérieux (France).

(2) Performed by Institut Mérieux (France).

(3) LT, thermolabile, tested with Y1 cells by D. Mathieu and with vascular permeability of rabbit skin ; ST, thermostable, tested with infant mice.

(4) Drig = Drigalski agar, lactose-containing medium ; DCA-Raf, modified deoxycholate agar medium supplemented with raffinose and neutral red for the indication of raffinose fermentation ; this medium was supplemented with rifampin (Rif) at 30 g/ml or with Tc, at 16 g/ml.

Details of the methods have been previously described (Duval *et al.*, 1981 and 1983).

Piglet inoculation. — Two-day old piglets were first inoculated with one of the two NETEC strains (EM0 or EM4) then, 24 h later, they were inoculated with one of the two ETEC strains (K88-positive or K88-deprived). They were given by mouth an inoculum of 2 ml of an 18-hour culture of *E. coli* in Trypticase Soy Broth (Difco Laboratories, Michigan) containing 10^9 viable cells.

Sampling. — The Large White piglets which were inoculated with both the K88-positive ETEC and EM4 strains were sacrificed with chloroform 18 h after inoculation with the ETEC strain. The other piglets were sacrificed no later than 17 days after inoculation with the ETEC strain, as described on table 4. Autopsies were performed either immediately after sacrifice or no later than 12 h after death when the piglets died during the night. Fifteen-cm long ligatured segments were removed from both the proximal end of the jejunum (S1) and the distal end of the ileum (S3). The intestine was then removed and unravelled and a third 15-cm long ligatured segment was taken from the midgut (S2). Contents from S1, S2 and S3 were removed. The segments were cut open longitudinally and the mucosal surface was exposed and generously washed with sterile PBS using a needle-free syringe until all visible traces of the lumen contents had disappeared.

Evidence of in vivo *adhesion of K88-positive* E. coli *by means of differential counts.* — The occurrence of specific attachment of K88-positive *E. coli* to intestinal epithelial tissues was assessed by means of differential bacterial counts. Bertschinger *et al.* (1972) have shown that, despite repeated washing of the intestinal walls, some residues originating from the luminal contents cannot be completely removed. Therefore, in the case of adhesion-susceptible piglets one may assume that the total number, X, of the K88-positive *E. coli* numerated on

the washed intestinal walls included a number of bacteria, Xa, specifically attached to the epithelial walls, and a second number of bacteria, Xr, from the luminal residues (X = Xa + Xr). Conversely, in the case of adhesion-resistant piglets, X would exclusively represent the luminal residues. Since we could not directly assess the population levels of Xa and Xr by bacterial counts, we inoculated the piglets with a second strain of *E. coli* which lacked the K88 antigen and constituted an inner marker. The population level, Yr, of the latter strain on the washed walls was directly determined by differential counts. The population levels of K88-positive and K88-deprived *E. coli* strains in the luminal contents were designated as x_c and y_c, respectively, and their ratio as $\frac{x_c}{y_c} = \rho$. The ratio between the population levels of K88-positive and K88-deprived *E. coli* strains on washed walls was designated as $P = \frac{X}{Y_r}$. The ratios P and ρ could be directly calculated from the bacterial counts. Since Xr and Yr came from the luminal contents, it was assumed that $\frac{Xr}{Yr} = \frac{x_c}{y_c} = \rho$. Therefore, $Xr = \rho Yr$ and $P = \frac{Xa}{Yr} + \rho$. The number of specifically attached bacteria could thus be expressed as $Xa = Yr\,(P - \rho)$. The evidence of adhesion was assessed by the ratio Γ between Xa and the total number Xr + Yr which came from the luminal residues. Thus, $\Gamma = \frac{Xa}{Xr + Yr} = \frac{P - \rho}{\rho + 1}$. Since bacterial counts follow a Poisson distribution, a confidence interval $\Gamma \pm \Delta\Gamma$ was calculated for each Γ value.

Statistical analysis. — The population levels of the various *E. coli* strains in the intestinal lumen contents and on the walls were compared by one-way analysis of variance for independent samples and by the t-test of differences between pairs for correlated samples.

Immunofluorescence test for attachment (IF test). — In three piglets taken at random, serial 0.4-µm sections were performed on frozen portions of intestine adjacent to each of the S1, S2 and S3 segments. K88-positive bacteria were labelled by an indirect immunofluorescence technique according to the method described by Arbuckle (1970). The controls were performed using antisera directed against strains EM4 or EM0.

Results.

1. *Distribution of gnotobiotic Large White and Chinese piglets associated with various strains of* E. coli.

The piglets were distributed into four groups according to mean Γ values (ΓM) and to Γ confidence intervals as indicated on figures 1 and 2 and summarized on table 2. In the first experiment, the Γ ratios were calculated for 8

TABLE 2

Characteristics of the 4 groups of gnotobiotic piglets studied.

Group (no of animals)	Breed	Inoculated *E. coli* strain	ΓM (1)	Confidence interval of Γ ratio (1)	
				Γ − ΔΓ	Γ + ΔΓ
1 (8)	Large White	K88-negative ETEC + EM4	< 3	< 3	< 8
2 (8)	Large White	K88-positive ETEC + EM4	> 6	> 8	> 24
3 (7)	Large White	K88-positive ETEC + EM4	< 2	< 2	< 5
4 (10)	Chinese	K88-positive ETEC + EM0	< 4	< 3	< 9

(1) See figs. 1 and 2 for the definition of ΓM, Γ + ΔΓ and Γ − ΔΓ.

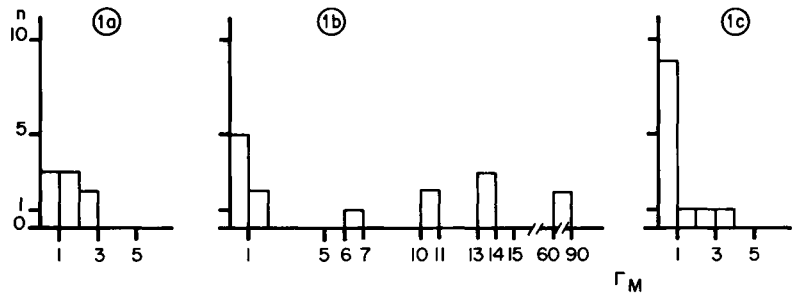

FIG. 1. — *Distribution of gnotobiotic Large White and Chinese piglets inoculated with various strains of* E. coli *according to the* ΓM *values.*
1a : Large White piglets inoculated with K88-deprived ETEC and EM4 strains.
1b : Large White piglets inoculated with K88-positive ETEC and EM4 strains.
1c : Chinese piglets inoculated with K88-positive ETEC and EMO strains.
n = Number of inoculated piglets.
ΓM = mean value of the three Γ ratios calculated for each piglet.

Large White piglets inoculated with the two K88 antigen-deprived *E. coli* strains, EM4 and K88-negative ETEC. Neither of these strains could attach to the intestinal wall. In this experiment, the numbers X and x_c represented the population levels of K88-negative ETEC on the washed wall and in the contents, respectively. Figure 1a indicates the ΓM of the three Γ ratios calculated from the three intestinal segments which were removed from each one of the group 1 piglets described on table 2 ; figure 2a indicates the values of $\Gamma \pm \Delta\Gamma$. All the ΓM and the $\Gamma - \Delta\Gamma$ values were < 3, and all the $\Gamma + \Delta\Gamma$ values were < 8.

In the second experiment, 15 Large White piglets were inoculated with both the K88 antigen-positive and the marker *E. coli* strain EM4. Figure 1b shows that these piglets were distributed into groups 2 and 3 according to their ΓM values. In group 2 (8 animals), the ΓM values were always > 6 (fig. 1b) ; in group 3 (7 animals), the ΓM values were always < 2 (fig. 1b). The $\Gamma - \Delta\Gamma$ and $\Gamma + \Delta\Gamma$ values of 7 out of 8 piglets of group 2 were always > 8 and > 24, respectively, whereas they were 6 and 28, respectively, for the remaining piglet. These values were < 2 and < 5, respectively, in the group 3 piglets (fig. 2b).

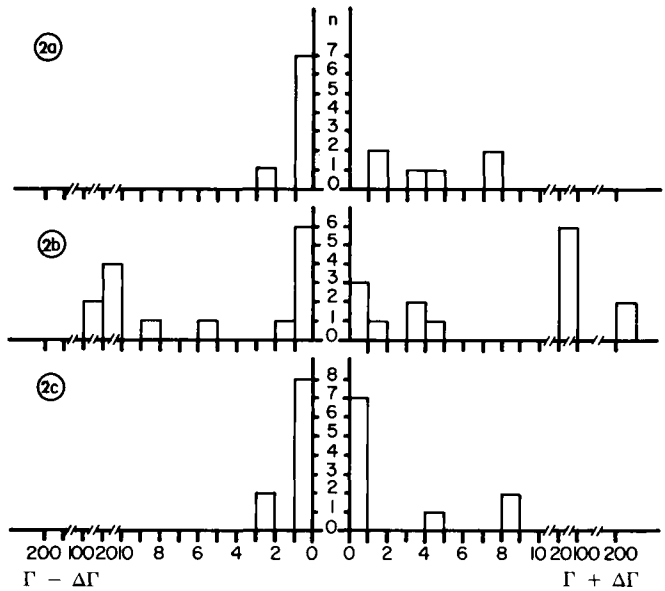

FIG. 2. — *Distribution of gnotobiotic Large White and Chinese piglets inoculated with various strains of* E. coli *according to the confidence interval of the* Γ *value.*
2a : Large White piglets inoculated with K88-deprived ETEC and EM4 strains.
2b : Large White piglets inoculated with K88-positive ETEC and EM4 strains. Individual values of $\Gamma + \Delta\Gamma$ above 20 were : 24, 28, 31, 35, 41, 60, 237, 303. Individual values of $\Gamma - \Delta\Gamma$ above 10 were : 12, 15, 15, 17, 25, 95.
2c : Chinese piglets inoculated with K88-positive ETEC and EM0 strains.
n : Number of inoculated piglets.
$\Gamma + \Delta\Gamma$ and $\Gamma - \Delta\Gamma$ are the maximal values at the upper limit and the lower limit, respectively, of the Γ confidence interval over the 3 segments of the individual piglets.

In the third experiment, 10 Chinese piglets were inoculated with both K88-positive ETEC and with the marker strain EM0. Figure 1c shows that the ΓM values were always < 4. Figure 2c shows that the $\Gamma \pm \Delta\Gamma$ values of 7 out of 10 Chinese piglets ranged between 0 and 1, whereas the confidence interval of the remaining piglets did not exceed 9.

2. *Comparative population levels of the various* E. coli *strains in the luminal contents and on the walls of the intestinal segments of the 4 groups of gnotobiotic piglets.*

We carried out one-way analysis of variance of the independent data reported on table 3 and a t-test of the differences between the population levels of both the strains present in the same piglets. The wall population levels of all the K88-negative strains (*i.e.* K88-deprived ETEC, EM4 and EM0) were always $< 10^8$ CFU/g and not significantly different ($P > 0.05$) in the three segments of any of the tested piglets, whatever group they belonged to. The wall population levels of the K88-positive ETEC strain were also $< 10^8$ CFU/g in group 3 and 4 piglets. In the latter piglets, the population levels of the K88-

positive ETEC strain on the walls were not significantly different ($P > 0.05$) from those of the K88 antigen-deprived strains, except for segment S3 in group 4 where the K88-positive ETEC was at a disadvantage ($P < 0.05$). In contrast, the population levels of the K88-positive ETEC strain on the walls of group 2 piglets were most often $> 10^8$ CFU/g and significantly higher ($P < 0.01$) than those of the same strain on the walls of group 3 and 4 piglets.

According to the t-test, the mean population levels of both the EM4 and EM0 strains were 10 to 100-fold less, or significantly lower, on the walls than in the luminal contents in the three segments of the four groups ($P < 0.01$). The same results were obtained for both K88-positive and K88-negative ETEC strains in the three segments of group 1, 3 and 4 piglets (individual data not shown). In contrast, the mean values of the population levels of the K88-positive ETEC strain were not significantly different ($P > 0.05$) in the contents and on the walls of the three segments of group 2 piglets (individual data not shown). Moreover, neither of the population levels of the ETEC or the EM4 strains was significantly different ($P > 0.05$) in group 2^a piglets which died within 18 h after association with the ETEC strain or in group 2^b piglets which were sacrificed 18 h after inoculation with the ETEC strain (table 3). In the contents of group 2 piglets, the K88-positive ETEC strain was at an advantage over the marker EM4 strain ($P < 0.05$). On the contrary, the former strain was at a disadvantage compared to the EM0 marker strain ($P < 0.05$) in the contents of group 4 piglets. No significant differences ($P > 0.05$) were observed between the content levels of either the ETEC or EM4 strains in the three segments of group 1 and 3 piglets, whatever the age of the animals.

TABLE 3

Comparative population levels of the various E. coli *strains in the luminal contents and on the walls of intestinal segments S1, S2 and S3 in 4 groups of gnotobiotic piglets* ([1]).

Group	*E. coli* strain		Mean $\log_{10}$ (SD) CFU/g of luminal contents			Mean $\log_{10}$ (SD) CFU/g of walls		
			S1	S2	S3	S1	S2	S3
1	K88$^-$ ETEC		8.2 (0.9)	7.9 (1.0)	8.9 (0.6)	6.9 (0.8)	6.6 (1.5)	7.3 (0.3)
	EM4		8.2 (0.5)	8.1 (1.0)	8.3 (0.7)	6.7 (0.5)	6.3 (1.0)	6.9 (0.3)
2 ([2])	K88$^+$ ETEC	a	8.6 (0.7)	9.0 (0.2)	8.6 (0.4)	8.1 (1.1)	8.8 (0.6)	8.5 (0.8)
		b	8.5 (0.7)	8.8 (0.3)	8.6 (0.5)	7.6 (1.2)	8.3 (0.7)	8.0 (0.7)
	EM4	a	6.3 (1.1)	7.2 (1.1)	7.6 (0.8)	4.7 (1.0)	6.4 (1.5)	6.5 (1.2)
		b	7.3 (0.4)	7.7 (0.8)	7.6 (1.2)	5.8 (1.5)	6.4 (0.7)	6.0 (0.8)
3	K88$^+$ ETEC		6.4 (1.7)	7.9 (0.6)	8.2 (0.9)	4.9 (1.5)	6.4 (1.2)	6.5 (1.5)
	EM4		6.6 (1.6)	8.3 (0.9)	8.4 (1.6)	5.1 (1.3)	6.9 (0.9)	6.8 (1.4)
4	K88$^+$ ETEC		6.4 (1.4)	6.8 (1.3)	8.0 (1.1)	4.9 (1.3)	4.7 (1.3)	5.5 (0.9)
	EM0		7.6 (1.3)	7.8 (1.6)	9.2 (0.6)	5.6 (1.8)	5.8 (1.0)	6.6 (0.8)

([1]) The groups are defined in table 2 ; S1 = proximal end of jejunum ; S2 = midgut ; S3 = distal end of ileum ; SD = standard deviation ; CFU = colony-forming units.

([2]) Group 2a = 4 piglets which died within 18 h of association with ETEC strain. Group 2b = piglets which were sacrificed 18 h after inoculation with ETEC strain.

IF test. — The IF test was performed on three piglets taken at random, including 1 piglet of group 1 as a control, 1 piglet of group 2 and 1 piglet of group 4. Using antiserum directed against strains EM4 and EM0, no fluorescence was observed adjacent to the villi. In contrast, fluorescence was observed closely associated to villi with K88 antiserum in sections from segments S1 and S2 of the group 2 piglet. There was no fluorescence adjacent to the villi in the group 4 Chinese piglet nor in the group 1 control piglet.

3. *Health status of the gnotobiotic piglets before autopsy.*

Table 4 shows that, within 18 h after association with the ETEC strain, 5/8 of the group 2 piglets were dead or dying, whereas none of the group 3 piglets were dead. The piglets of groups 1 and 4 were healthy 18 h after inoculation with the ETEC strain. Among the group 1 piglets, three were dead or dying at day 4 and three at days 5, 10 and 17. The remaining two piglets were healthy at autopsy which was performed at days 13 and 14. Among the group 4 piglets, two were dead or dying at day 5 and one at day 7. The remaining 7 piglets were healthy at autopsy which was performed at day 1 for three piglets and at days 2, 4, 7 and 9 for the other four.

TABLE 4

Health status of the tested gnotobiotic piglets 18 h after inoculation with a K88-positive of K88-negative strain.

Group ([1]) (number of piglets)	K88 status of the inoculated ETEC strain	Dead or dying	Diarrheic	Healthy
1 (8)	$K88^-$	0	0	8
2 (8)	$K88^+$	5	2	1
3 (7)	$K88^+$	0	2	5
4 (10)	$K88^+$	0	0	10

([1]) These groups are described on table 2.

Discussion.

From the results, it is apparent that it was advantageous to use a non-adhesive strain as a marker for demonstrating the adhesive or non-adhesive phenotype of the gnotobiotic piglets. According to the distribution of both the ΓM and $\Gamma \pm \Delta\Gamma$ values, Large White piglets challenged with the K88-positive ETEC and marker strains were classified into two distinct groups. The piglets of group 3 were obviously similar to those of group 1 in which the K88-positive ETEC strain was replaced by its K88-deprived derivative. Consequently, the piglets of group 3 belonged to the non-adhesive phenotype. The characteristics of groups 1 and 3 were a Γ ratio close to 0, and wall population levels of both the ETEC and marker strains $< 10^8$ CFU/g and 10 to 100-fold lower than their corresponding levels in the luminal contents.

On the contrary, group 2 Large White piglets differed extensively from those of groups 1 and 3. The Γ ratio was always far from 0, wall population levels of the K88-positive strain were $> 10^8$ CFU/g in at least two out of the three tested segments and similar to those in the contents. Conversely, wall population levels of the marker strain were always $< 10^8$ CFU/g in the three segments and 10-fold lower than in the contents. Therefore, group 2 piglets were shown to belong to the adhesive phenotype. In addition, a positive IF test was evidenced on segments S1 and S2 in one piglet of group 2.

Group 4 Chinese piglets exhibited the same characteristics as those of groups 1 and 3. Consequently, they belonged to the non-adhesive phenotype. The same conclusion may be drawn from the IF test which was negative in one piglet each of groups 1 and 4, respectively.

In our experiments, 7/15 Large White piglets belonged to the non-adhesive phenotype. This frequency is consistent with those reported by other authors (Walters and Sellwood, 1982 ; Edfors-Lilja *et al.,* 1982 ; Snodgrass *et al.,* 1981).

The fact that all our Chinese piglets belonged to the non-adhesive phenotype suggests the existence of a resistance to attachment among some Chinese breeds. However, firm conclusions cannot be drawn as to the resistant status of the two Chinese breeds investigated because of the limited sample size and the restricted within-breeding sampling since our Chinese piglets came from one sire and two dams of the Meishan and Jiaxing breeds, respectively. In addition, only one serological K88ab variant was used. Bijlsma *et al.* (1981) showed the existence of five piglet phenotypes in relation with the three serological K88 antigen variants. Group A was adhesion-susceptible to K88ab, ac and ad variants, whereas group E was adhesion-resistant to these three variants. Groups B and C were adhesion-resistant to only K88ad and ac, respectively, and group D was adhesion-susceptible to only K88ad. Accordingly, our Chinese piglets belonged to the D or E phenotype, and thus might still be susceptible to the K88ad variant.

The enteropathogenicity of K88-positive ETEC strains has been widely demonstrated. Our results show that the C5148 K88-positive strain we used was also highly enteropathogenic since 7 out of 8 « adhesive » piglets challenged with this strain were dead or diarrheic within 18 h after association. In contrast, none of the non-adhesive piglets died within 18 h in the same conditions. However, 3/10 Chinese, non-adhesive piglets died within 5 and 7 days after inoculation with this strain, as did 6/8 Large White piglets inoculated with the K88-deprived ETEC strain. Several authors (Duval *et al.,* 1983 ; Jones and Rutters, 1974 ; Miniats and Gyles, 1972) have already demonstrated the enteropathogenicity of K88-deprived strains in gnotobiotic Large White piglets. The fact that the survival times of some group 1 and 4 piglets were protracted might be due to the protective effect of the associated NETEC strains, as already demonstrated by Duval *et al.,* (1983). In the conventional piglets, a similar barrier effect might be realized by other NETEC strains of *E. coli* which spontaneously become established at birth and therefore protect the piglets against the enteropathogenicity of the K88-negative ETEC strains. This might explain why the latter strains are not considered as ethiological agents in the diarrhea of conventional piglets.

Whatever the health status of group 1 and 4 piglets and the duration of their association with the ETEC strains, their characteristics were the same as those of group 3 non-adhesive piglets, *i.e.* a Γ value close to 0, wall population levels $< 10^8$ CFU/g and 10 to 100-fold lower than in the luminal contents, even though they were autopsied later than 18 h after infection with the ETEC strains. Since the piglets of groups 1 and 4 were sacrificed or dead at different times after infection with the K88-positive or K88-negative ETEC strains, these results show that the duration of association with either of the strains did not markedly alter the relative bacterial counts in the small intestine. Our results also show that group 4 Chinese piglets were sensitive to the enterotoxin produced by the K88-positive ETEC strain since three of them died with obvious signs of enteritis. If group 4 piglets had belonged to the « adhesive » phenotype, most of them would have died 18 h after infection with the K88-positive ETEC strain, as did the group 2 piglets.

With regard to the luminal contents, it was noticeable that the population levels of both the K88-positive and K88-deprived ETEC strains were similar in all the Large White piglets, whatever group they belonged to. This suggests that adhesion to the intestinal wall did not significantly increase the population level of the K88-positive ETEC strain in the luminal contents of gnotobiotic piglets. This result is in agreement with those of Miniats and Gyles (1972) and Jones and Rutter (1972). On the other hand, the luminal population levels of the K88-positive ETEC strain in segments S2 and S3 of group 4 Chinese piglets were significantly lower than in the corresponding segments of the Large White piglets. This might be due to an efficient antagonism exerted by the marker EMO strain against the ETEC strain.

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Résumé. *Résistance de porcelets gnotoxéniques de races chinoise et Large White à l'attachement in vivo d'une souche de* Escherichia coli *entérotoxinogène K88ab.*

L'adhésion *in vivo* d'une souche de *Escherichia coli* entérotoxinogène (ECET) possédant l'antigène d'attachement K88ab à la paroi de l'intestin grêle a été étudiée chez des porcelets gnotoxéniques de races chinoise et Large White, privés de colostrum. Pour déterminer les résidus de contenus intestinaux sur les parois de ces porcelets, une souche témoin de *E. coli* non entérotoxinogène et dépourvue d'antigène d'attachement a été inoculée avec la souche ECET K88ab aux porcelets gnotoxéniques. Les deux souches ont été dénombrées sélectivement à la fois dans le contenu et sur la paroi lavée de trois segments de l'intestin grêle. L'attachement *in vivo* a été défini par le rapport Γ entre le nombre de ECET K88ab spécifiquement attachés aux parois et le nombre total des deux souches $K88^+$ et $K88^-$ provenant des résidus de contenu intestinal qui n'a pu être éliminé par lavage.

Ce rapport Γ a d'abord été calculé sur un groupe de 8 porcelets Large White inoculés avec la souche témoin et une souche fille de la souche entérotoxinogène qui a perdu l'anti-

gène d'attachement K88ab. Nous avons ainsi défini les limites de variation des rapports Γ chez des porcelets inoculés avec deux souches de *E. coli* dépourvues de facteurs d'attachement. La comparaison des rapports précédents avec ceux calculés chez 15 porcelets Large White et 10 porcelets chinois inoculés avec la souche témoin et la souche ECET K88ab nous a ensuite permis de classer les porcelets Large White en deux groupes distincts, l'un de phénotype résistant (7 porcelets), l'autre de phénotype sensible (8 porcelets), et de montrer que le phénotype des 10 porcelets chinois était du type résistant.

References

ARBUCKLE J. B. R., 1970. The localization of *Escherichia coli* in the pig intestine. *J. med. Microbiol.*, **3**, 333-340.

BERTSCHINGER H. U., MOON H. W., WHIPP S. C., 1972. Association of *Escherichia coli* with the small intestinal epithelium. I. Comparison of enteropathogenic and nonenteropathogenic porcine strains in pigs. *Infect. Immun.*, **5**, 595-605.

BIJLSMA I. G. W., DENIJS A., FRIK J. F., 1981. Adherence of *Escherichia coli* to porcine intestinal brush borders by means of serological variants of the K88 antigen. Antonie van Leeuwenhoek. *J. Microbiol. Serol.*, **47**, 467-468.

DUCLUZEAU R., RAIBAUD P., LAUVERGEON B., GOUET Ph., RIOU Y., GRISCELLI C., GHNASSIA J. C., 1976. Immediate postnatal decontamination as a means of obtaining axenic animals and human infants. *Can. J. Microbiol.*, **22**, 563-566.

DUVAL-IFLAH Y., CHAPPUIS J. P., DUCLUZEAU R., RAIBAUD P., 1983. Intraspecific interactions between *Escherichia coli* strains in human newborns and in gnotobiotic mice and piglets. *Prog. Fd. Nutr. Sci.*, **7**, 107-116.

DUVAL-IFLAH Y., RAIBAUD P., ROUSSEAU M., 1981. Antagonisms among isogenic strains of *Escherichia coli* in the digestive tracts of gnotobiotic mice. *Infect. Immun.*, **34**, 957-969.

EDFORS-LILJA I., PETERSSON H., GAHNE B., 1982. The intestinal receptor for *E. coli* K88 in relation to performance of sw. Yorkshire x sw. Landrace pigs. In *2nd World Congr. on Genetics applied to Livestock Production.* Vol. **VII**, 390-394, Editorial Garsi, Madrid.

GUINEE P. A. M., JANSEN W. H., 1979a. Behavior of *Escherichia coli* K antigens K88ab, K88ac and K88ad in immunoelectrophoresis, double diffusion and hemagglutination. *Infect. Immun.*, **23**, 700-705.

GUINEE P. A. M., JANSEN W. H., 1979b. Detection of enterotoxigenicity and attachment factors in *Escherichia coli* strains of human, porcine and bovine origin : a comparative study. *Zbl. Bakt. Hyg. I. Abt. Orig. A*, **243**, 245-257.

JONES G. W., RUTTER J. M., 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhae caused by *Escherichia coli* in piglets. *Infect. Immun.*, **6**, 918-927.

JONES G. W., RUTTER J. M., 1974. Contribution of the K88-antigen of *Escherichia coli* to enteropathogenicity ; protection against disease by neutralizing the adhesive properties of K88-antigen. *Am. J. clin. Nutr.*, **27**, 1441-1449.

LEGAULT C., CARITEZ J. L., 1982. First experiments with Chinese pigs in France. 1. Reproductive performance in pure and cross breeding with European breeds. *In Journées Rech. porcine en France,* 127-136, I. T. P. ed., Paris.

MINIATS O. P., GYLES C. L., 1972. The significance of proliferation and enterotoxin production by *Escherichia coli* in the intestine of gnotobiotic pigs. *Can. J. comp. Med.*, **36**, 150-159.

MOON H. W., KOHLER E. M., SCHNEIDER R. A., WHIPP S. C., 1980. Prevalence of pilus antigens, enterotoxin types and enteropathogenicity among K88-negative enterotoxigenic *Escherichia coli* from neonatal pigs. *Infect. Immun.*, **27**, 222-230.

ØRSKOV I., ØRSKOV F., SOJKA W. J., WITTIG W., 1964. K antigens K88ab (L) and K88ac (L) in *E. coli.* A new O antigen : 0147 and a new K antigen : K89 (B). *Acta pathol. microbiol. scand., Sect. B,* **62**, 439-447.

RUTTER J. M., BURROWS M. R., SELLWOOD R., GIBBONS R. A., 1975. A genetic basis for resistance to enteric disease caused by *E. coli. Nature (London),* **257**, 135-136.

SELLWOOD R., 1980. The interaction of the K88 antigen with porcine epithelial cell brush borders. *Biochim. biophys. Acta,* **632,** 326-335.

SELLWOOD R., GIBBONS R. A., JONES G. W., RUTTER J. M., 1975. Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders : the existence of two pig phenotypes. *J. med. Microbiol.,* **8,** 405-411.

SMITH H. W., HUGGINS M. B., 1978. The influence of plasmid-determined and other characteristics of enteropathogenic *E. coli* on their ability to proliferate in the alimentary tracts of piglets, calves and lambs. *J. med. Microbiol.,* **11,** 471-492.

SMITH H. W., LINGOOD M. A., 1971. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *E. coli* with particular reference to porcine diarrhoea. *J. med. Microbiol.,* **4,** 467-485.

SMITH H. W., PARSELL Z., 1975. Transmissible substrate utilizing ability in enterobacteria. *J. gen. Microbiol.,* **87,** 129-140.

SNODGRASS D. R., CHANDLER D. A., MAKIN T. J., 1981. Inheritance of *Escherichia coli* K88 adhesion in pigs : identification of nonadhesive phenotypes in a commercial herd. *Vet. Rec.,* **109,** 461-463.

SÖDERLIND O., MÖLLBY R., 1979. Enterotoxins, O-groups and K88 antigen in *Escherichia coli* from neonatal pigs with and without diarrhae. *Infect. Immun.,* **24,** 611-616.

SOJKA W. J., 1971. Enteric diseases in new-born piglets, calves and lambs due to *Escherichia coli* infection. *Vet. Bull.,* **41,** 509-522.

STIRM S., ØRSKOV F., ØRSKOV I., BIRCH-ANDERSEN A., 1967. Episome-carried surface antigen K88 of *Escherichia coli.* III. Morphology. *J. Bacteriol.,* **93,** 740-748.

WALTERS J. R., SELLWOOD R., 1982. Aspects of genetic resistance to K88 *E. coli* in pigs. In *2nd World Congr. on genetics applied to Livestock Production.* Vol. **VII,** 362-367. Editorial Garsi, Madrid.